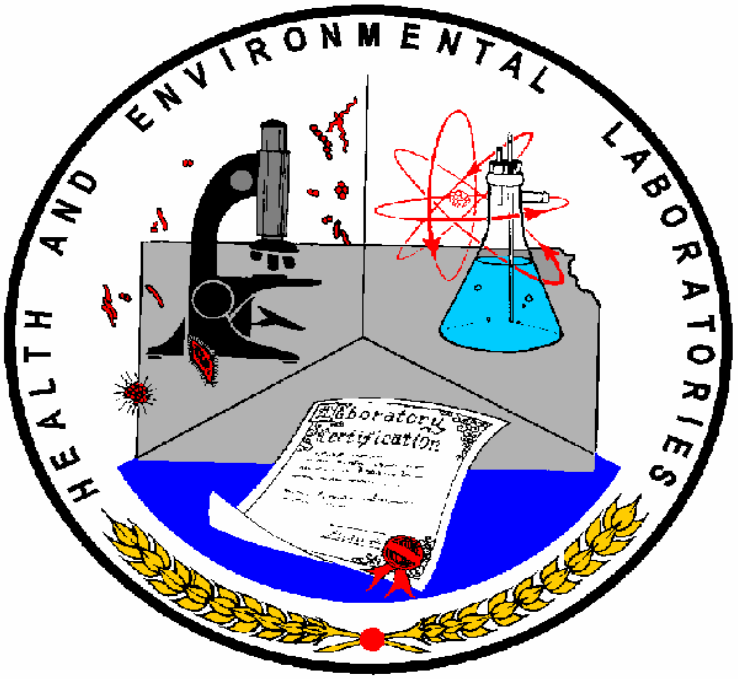
MANUAL OF HEALTH LABORATORY TESTS

DIVISION OF HEALTH AND ENVIRONMENTAL LABORATORIES



KANSAS DEPARTMENT OF HEALTH AND ENVIRONMENT

September 2004

CURRENT LABORATORY STAFF AND TELEPHONE NUMBERS

Laboratory Main Number.....(785) 296-1620
facsimile (785) 296-1641

Laboratory Administration ..... Duane Boline, Ph.D., Director

Quality Assurance and Laboratory Improvement .....Richard Howard, B.S., D.C.

Sample and Data Management.....Robert Bostrom, M.S.

Neonatal Screening.....Willie Craft, M.S.

Microbiology ..... Robert Flahart, Ph.D.

Virology and Serology ..... Patrick Hays, Ph.D.

Environmental Chemistry ..... Jon Brady, B.S.

Bioterrorism Program ..... Sue Riley, B.S., CLS (NCA)

Chemical Terrorism Program .....Julia Hanson, B.S.

LABORATORY MANUAL COMMITTEE

Patrick Hays, Ph.D.	Sue Riley, B.S., CLS (NCA)
Willie Craft, M.S.	Robert Bostrom, M.S.
Robert Flahart, Ph.D.	Richard Howard, B.S., D.C.

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LINKS TO FORMS OF INTEREST:

1.) Copy of CDC 50.34 Form
www.cdc.gov/ncidod/dvbid/westnile/resources/cdc_form5034.pdf

2.) Current List of Reportable Diseases in Kansas

www.kdhe.state.ks.us/disease_reporting/download/Ks_disease_report_form.pdf

3.) Requisition for Laboratory Specimen Kits
www.kdhe.state.ks.us/sdm/download/specimen_kit_request_form.pdf

I. GENERAL INFORMATION

INTRODUCTION

The MANUAL of HEALTH LABORATORY TESTS is designed to assist laboratorians and clinicians in the collection and transportation of appropriate specimens for tests performed at the Division of Health and Environmental Laboratories (DHEL) or at CDC facilities. Conscientious usage of this manual will result in **APPROPRIATE, ADEQUATE, TIMELY**, and **TRACEABLE** specimens.

GENERAL INFORMATION

LOCATION AND POSTAL ADDRESS:

Division of Health and Environment Laboratories
Kansas Department of Health and Environment
Forbes Field Building #740
Topeka, Kansas 66620-0001

Alternate Shipping Address:

Division of Health and Environmental Laboratories
Kansas Department of Health and Environment
6700 SW Topeka Blvd, Building #740
Topeka, Kansas 66620-0001

Telephone: (785) 296-1620

OFFICIAL BUSINESS HOURS: 8:00 a.m. through 5:00 p.m., Monday through Friday.

ANNUAL HOLIDAY SCHEDULE: The state public health laboratory observes: New Year's Day, Martin Luther King Day, Memorial Day, Independence Day, Labor Day, Veteran's Day, Thanksgiving Day and the following day, and Christmas Day. Holidays which fall on Saturday are observed on the preceding Friday. Holidays which fall on Sunday are observed on the following Monday.

PARKING:

Pick-up and Delivery -- Truck loading dock on north side of building
Visitor Parking -- Spaces in front of building (west side)

DELIVERY OF SPECIMENS

Postal Services: Daily except Sunday (specimens arriving on Saturday are held until Monday). Special handling services available through Postal Service for critical items. (On federal holidays, mail will not be delivered.)

UPS: Monday through Friday

Private Courier Service: Monday through Saturday
In case of emergency obtain Hot Shot authorization for terrorism or other emergency samples from KDHE Epidemiology at the toll free number at (877) 427-7317.

Delivery in Person: During business hours, bring specimens to first floor receiving area adjacent to loading dock. After working hours, weekends, and holidays, telephone to establish that a security guard will be available to accept the specimen/sample at (785) 368-6499.

LABORATORY SERVICES

Bioterrorism: The laboratory will perform confirmatory testing on any suspected bio-terrorism isolate submitted by any clinical laboratory throughout the state. The laboratory can also test nonclinical specimens for agents of bio-terrorism. However these specimens must be screened by law enforcement and the KDHE Epidemiologic Services section (toll free 877- 427-7317) before being accepted. For more information on
bio-terrorism, bio-terrorism testing, and bio-terrorism specimen submission see the KDHE web site at www.kdhe.state.ks.us and the Centers for Disease Control and Prevention web site at www.bt.cdc.gov.

Index of Laboratory Tests: A listing of tests available from the Division of Health and Environmental Laboratories, and a quick reference for best choice of specimen, volume required, and mailing information for specific tests is shown in the Index section of the manual.

Specimen Kits and Containers: This section provides descriptions of Division of Health and Environmental Laboratories-supplied material and special handling requirements needed to procure the specimens. **Mailers are not provided for shipping specimens elsewhere.**

Technical Information: This section details the available tests, special instructions for specific tests, and information on reporting procedures. These statements are designed to be concise. Please telephone if you require more detailed information.

This manual is provided for all users of the state public health laboratory.

MISSION AND OBJECTIVES

The health laboratory tests available at the Division of Health and Environmental Laboratories are targeted at the diagnosis and prevention of diseases of public health interest. These efforts are specifically designed to provide analytical support for public health programs in Kansas. This responsibility reflects both statutory requirements and administrative policies that are necessary to protect the health and environment of our state.

Several hundred thousand specimens are received each year from physicians, hospitals, clinical laboratories, local health departments and private citizens. Laboratory results are returned to the client to assist in the diagnosis, control, treatment, and prevention of disease. Additional efforts in technical training, consultation, certification, and proficiency testing are also provided to health and environmental laboratories throughout the state to help maintain and improve laboratory performance.

POLICIES AND LIMITATIONS

Specimens must be properly labeled with patient's name or another unique identifier using a waterproof ink to prevent smearing and washing off. Unidentified specimens will be considered unsatisfactory.

Each specimen must be accompanied by appropriate information. Specimen data forms are provided upon request. The information requested on these sheets is necessary for proper assessment and handling of each specimen. Any additional pertinent information which would facilitate rapid and definitive laboratory analysis should be included. Federal regulations require proper packing and labeling of infectious substances (e.g. culture isolates) transported by mail (see Appendix A). Suitable specimen containers must be used. Several specimen kits are available on request for some of the laboratory tests offered (see LABORATORY SPECIMEN KITS, p.24).

The Division of Health and Environmental Laboratories, in collaboration with public health officials, reserves the right to decide whether or not to analyze specimens. Contact the director or appropriate senior scientist before collecting or sending an unusual number of specimens (epidemics or surveys) to establish that the specimens can be analyzed or that sufficient selective medium can be made available.

Laboratory reports will be released only to the submitter of record or other authorized personnel. When it is necessary to call or inquire about test results, please ask for the Laboratory Sample and Data Management Office at (785) 296-1620. When urgency requires a telephone report as soon as analyses are completed, prominently indicate this request on the specimen form.

If you have any questions concerning storage of specimens, please contact the appropriate laboratory as is indicated in the INDEX OF TESTS (see p.12) section of this

manual. For urgent specimens also contact the laboratory indicated so that any special preparation to facilitate the specimen(s) can be made prior to its arrival.

CONSULTATION

Please direct general or policy questions to the director's office. Each section leader may be contacted about specific problems or to obtain information beyond the scope of this manual, such as explanation of results, etc. Feedback for the improvement of laboratory services is welcomed.

The use of brand names in this manual does not constitute endorsement.

II. INDEX OF TESTS

KEY TO ABBREVIATIONS USED IN INDEX

CDC	Centers for Disease Control and Prevention
KC-CBB	Kansas City Community Blood Bank
CSF	Cerebrospinal Fluid
NA	None available
AFB	Acid Fast Bacilli
ELISA	Enzyme-linked Immunosorbent Assay
FTA-ABS-DS	Fluorescent Treponemal Antibody Absorption
NAAT	Nucleic Acid Amplified Test
PCR	Polymerase Chain Reaction
QFA	Quantitative Fluorometric Assay
RFFIT	Rapid Fluorescent Focus Inhibition Test
TRF	Time-Resolved Fluoroimmunoassay
VFA	Visual Fluorometric Assay
VTM	Viral Transport Medium
NP	Nasopharyngeal

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Actinomyces culture (Anaerobes)	TELEPHONE FOR INFORMATION 785-296-1620	39	IDS	CDC
Adenovirus culture	Throat or Rectal swab	66	Viral Culture	
AFB (Acid Fast Bacilli)	SEE TUBERCULOSIS			
Amoeba (Direct Exam)	SEE INTESTINAL PARASITES			
Amoeba serology	Serum (3-5 ml)	59	Serology	CDC
Anaerobic bacteria	TELEPHONE FOR INFORMATION 785-296-1620	39	IDS	CDC
Anthrax	TELEPHONE FOR INFORMATION 785-296-1620	39	IDS	
Arbovirus culture	Brain tissue, clotted blood or CSF	59,67	Viral Culture	CDC
Arbovirus serology (See West Niles Virus and SLE virus)	Acute and convalescent sera or CSF 3-5 ml of each	55	Serology	
Arthropods of medical significance	TELEPHONE FOR INFORMATION 785-296-1620	43		
Aspergillus serology	Serum or CSF (3-5 ml)	59	Serology	CDC
Babesia microti	Blood (thick and thin smear on slide)	42		CDC
Babesiosis serology	Serum (3-5 ml)	59	Serology	CDC
Beta streptococcus grouping	Reference culture	39	IDS	
Blastomyces culture (Blastomycosis)	Suspect isolates sent to CDC	47	IDS	CDC
Blood Lead Filter Paper Spot	Fill 3 circles with blood one at a time	71	Blood Lead Spot	
Blood Lead Venous	Whole blood (3-5 ml)	71	Blood Lead Whole Blood	
Bordetella pertussis	NP Bacterial Swab or NP Swab in sterile tube for PCR Test	40	PCR Kit	
Botulism	TELEPHONE FOR INFORMATION - EPIDEMIOLOGIC SERVICES (SEE APPENDIX E) 877-427-7317			
Botulism, infant	TELEPHONE FOR INFORMATION - EPIDEMIOLOGIC SERVICES (SEE APPENDIX E) 877-427-7317			
Brucella culture	Blood culture or reference culture	39	IDS	
Brucella serology	Acute and convalescent sera 3-5 ml of each	59	Serology	CDC

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Calicivirus-like agents	Fresh stool (marble-sized) in Cary-Blair	66 (PCR)	Enteric	
California encephalitis Serology	SEE ENCEPHALITIS SEROLOGY	59	Serology	CDC
Campylobacter	Fresh stool (marble-sized) in Cary-Blair or culture isolate	33	Enteric	
Candida serology	Serum or CSF (3-5 ml)	59	Serology	CDC
Cat Scratch Fever	Serum (3-5 ml)	59	Serology	CDC
Chagas Disease serology (Trypanosoma cruzi)	Serum (3-5 ml)	59	Serology	CDC
Chancre, syphilis	TELEPHONE - BUREAU OF EPIDEMIOLOGY AND DISEASE PREVENTION 877-427-7317			
Chickenpox (VZV) culture	Vesicle scraping	66	Viral Culture	
Chickenpox (VZV) serology	Serum (3-5 ml)	54	Serology	
C. trachomatis culture or Chlamydia/GC NAAT	TELEPHONE FOR INFORMATION 785-296-1620	49	Specimen kit not provided by DHEL.	
Chlamydia serology (Tracoma-Psittacosis Group)	Acute and convalescent sera 3-5 ml of each	59	Serology	CDC
Cholera and Non-Cholera vibrios	TELEPHONE FOR INFORMATION 785-296-1620	33	IDS	
Clonorchis sinensis	SEE INTESTINAL PARASITES	42	Parasite	
Coccidioides culture	Suspect isolates sent to CDC	47	IDS	CDC
Congenital syphilis (FTA-DS-ABS)	Serum or blood (not cord blood)	63	Serology	
Coxsackie A virus culture	Throat swab, feces, or CSF	66	Viral Culture	
Coxsackie B virus culture	Throat swab, feces or CSF	66	Viral Culture	
Cryptococcus serology	Serum (antibody); serum or CSF (antigen) 3-5 ml of each	59	Serology	CDC

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Cryptosporidium	BY REQUEST ONLY--SEE INTESTINAL PARASITES	43	Parasite	
Cysticercosis serology	Serum (3-5 ml)	59	Serology	CDC
Cytomegalovirus (CMV) culture	Urine or throat swab (do not freeze specimen)	66	Viral Culture	
Dengue fever serology	Acute and convalescent sera (3-5 ml) of each	59	Serology	CDC
Dientamoeba fragilis	SEE INTESTINAL PARASITES	42	Parasite	
Diphtheria culture	TELEPHONE FOR INFORMATION 785-296-1620	39	NA	
Diphyllobothrium latum (fish tapeworm)	SEE INTESTINAL PARASITES	42	Parasite	
Direct Dark field (Treponema examination)	Not performed		NA	
Drug susceptibility testing, Enterics and TB only	Culture isolate Call for information	33,47	IDS	
Eastern equine encephalitis (EEE) culture	SEE ENCEPHALITIS, CULTURES	66	Viral Culture	
Eastern equine encephalitis (EEE) serology	SEE ENCEPHALITIS, SEROLOGY	59	Serology	CDC
Echinococcus granulosis serology	Serum (3-5 ml)	59	Serology	CDC
Echovirus culture	Throat swab, feces, SEE TECHNICAL INFORMATION	66	Viral Culture	
Encephalitis, viral culture	Throat swab, feces, SEE TECHNICAL INFORMATION	66	Viral Culture	
Encephalitis, viral serology (see West Niles Virus)	Acute and convalescent serum or CSF	53,59	Serology	
Endolimax nana	SEE INTESTINAL PARASITES	42	Parasite	
Entamoeba histolytica, hartmanni, coli	SEE INTESTINAL PARASITES	42	Parasite	
Entamoeba serology	Serum (3-5 ml)	59	Serology	CDC
Enteric culture (SEE TECH. INFO.)	Feces (marble-sized) in modified Cary-Blair or reference culture	34	Enteric	

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Enterobius vermicularis	SEE PINWORMS	42	Pinworm	
Enterovirus culture	Stool, throat swab or CSF	66	Viral Culture	
Epstein-Barr Virus (EBV) serology (SEE TECH. INFO.)	Acute and convalescent sera 3-5 ml of each	59	Serology	CDC
E. coli 0157:H7	SEE ENTERIC CULTURE	34	IDS	
Farmer's Lung (thermophilic actinomycetes)	Serum (3-5 ml)	59	Serology	CDC
Fasciola buski, F. hepatica, F. gigantica	SEE INTESTINAL PARASITES	42	Parasite	
Filariasis (microfilariae)	Blood (thick and thin smear)	42	NA	CDC
Food poisoning	CONTACT CO. HEALTH DEPT. OR EPIDEMIOLOGIC SERVICES 877-427-7317			
Forms, specimen	ORDER FROM MAILROOM 785-296-1620			
FTA-ABS	SEE SYPHILIS SEROLOGY	63		
Fungal culture	Not performed		NA	
Fungal serology	Acute and convalescent sera 3-5 ml of each	59	Serology	CDC
Galactosemia screening (Neonatal)	Filter paper (fill circles w/ blood) See Technical Information or Call 785-296-1620	27 (VFA)	Neonatal	
German Measles	SEE RUBELLA	56		
Giardia lamblia (Giardiasis)	SEE INTESTINAL PARASITES	42	Parasite	
Gonorrhea culture	Thayer-Martin plates in CO2 atmosphere	37	Gonorrhea	
Haemophilus influenza	Reference culture	39	IDS	
Hantavirus serology	Serum (3-5 ml) Call Epidemiol-ogical Services at 877-427-7317	59	Serology	CDC

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Hemoglobinopathy	Filter paper (fill circles with blood) SEE TECH. INFO. 785-296-1620	27 (IEF)	Neonatal	
Hepatitis A	Serum or whole blood (3-5 ml)	53	Serology	
Hepatitis B (HBsAG) (SEE TECH. INFO.)	Serum or whole blood (3-5 ml)	53	Serology	KC-CBB CDC
Hepatitis C	Serum or whole blood (3-5 ml	53	Serology	
Herpes Simplex Virus (HSV) culture (not serotyped)	Vesicular scrapings; genital swabs; CSF; brain biopsy	66	Viral Culture	
Heterphyidae, flukes (not speciated)	SEE INTESTINAL PARASITES	42	Parasite	
Histoplasma culture	Suspected isolates sent to CDC	47	IDS	CDC
Hookworm (not speciated)	SEE INTESTINAL PARASITES	42	Parasite	
Hypothyroid screening (Neonatal)	Filter paper (fill circles with blood) SEE TECH. INFO. 785-296-1620	27 (TRF)	Neonatal	
Iodamoeba butschlii	SEE INTESTINAL PARASITES	42	Parasite	
Infectious Mononucleosis (EBV) serology	Serum (3-5 ml)	59	Serology	CDC
Influenza, viral culture	Nasopharyngeal swab, Throat swab	66	Viral Culture	
Influenza, viral serology	Not available		NA	
Intestinal parasites	Feces (marble-sized, mixed well in 10% formalin and PVA bottles)	42	Parasite	
Legionella sp.	Culture isolate	39	IDS	CDC
Legionella pneumophila serology	Acute and convalescent sera 3-5 ml of each	59	Serology	CDC
Leishmaniasis	Blood (thick and thin smear) ulcer on skin scraping	43	NA	CDC
Leishmaniasis serology1	Serum (3-5 ml)	59	Serology	CDC
Leptospirosis culture	TELEPHONE FOR INFORMATION 785-296-1620	40	NA	

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Leptospirosis serology1	Acute and convalescent sera 3-5 ml of each	59	Serology	CDC
LGV serology (Lymphogranuloma venereum)	Acute and convalescent sera 3-5 ml of each	59	Serology	CDC
Lice and mites, human	TELEPHONE FOR INFORMATION - Epidemiologic Services 877-427-7317			
Listeriosis	Culture isolate	39	IDS	
Lyme disease	Acute and convalescent serum 3-5 ml of each. Call for information 785-296-1620	59	Serology	CDC
Mailer Kits	ORDER FROM MAILROOM 785-296-1620	23		
Malaria, serology	TELEPHONE FOR INFORMATION - Epidemiologic Services 877-427-7317	59	Serology	CDC
Malaria, smear	SEE TECH. INFO.	43,59	NA	CDC
Measles, culture	SEE RUBEOLA, CULTURE	66	Viral Culture	
Measles, serology	SEE RUBEOLA, CULTURE	53	Serology	
Meningoencephalitis, amoebic	Serum (3-5 ml)	59	Serology	CDC
Mononucleosis	SEE INFECTIOUS MONONUCLEOSIS	59	Serology	CDC
Mumps, culture	Throat swab, CSF, urine (5-10 ml)	66	Viral culture	
Mumps, serology	Acute and convalescent sera 3-5 ml of each	53	Serology	
Murine typhus	Acute and convalescent sera 3-5 ml of each	53	Serology	
Mycobacterium	SEE TUBERCULOSIS	45		
Mycology culture	Not performed	47	NA	
Mycoplasma serology	Not available, Call for information 785-296-1620	59	NA	CDC

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Myocarditis serology SEE TECH. INFO.	Acute and convalescent sera (3-5 ml each) if positive culture from patient available	53	Serology	
Neurosyphilis	CSF (1-2 ml) Call For Information 785-296-1620	63	Serology	
Nocardia, culture	Call For Information (Genius ID Only) 785-296-1620	39	IDS	CDC
Nocardia, serology	Serum (3-5 ml)	59	Serology	CDC
Ova and Parasites	SEE INTESTINAL PARASITES	42	Parasite	
Paracoccidioidomycosis	Serum (3-5 ml)	59	Serology	CDC
Paragonimus westermani	Feces (SEE INTESTINAL PARASITES). Sputum. (Do not use PVA bottle)	42	Parasite	
Paragonimus westermani serology	Serum (3-5 ml)	59	Serology	CDC
Paratyphoid fever	SEE ENTERIC CULTURE	34	Enteric	
Pertussis	SEE BORDETELLA PERTUSSIS	40	PCR	
Phenylketonuria (PKU)	Filter paper (fill circles with blood) See Tech. Info. or Call 785-296-1620	27 (QFA)	NEONATAL	
Pinworms	Health Depts. only, SEE TECH. INFORMATION	42	PINWORM	
Plague	TELEPHONE FOR INFORMATION 785-269-1620	39	IDS	
Pneumocystis carinii serology	TELEPHONE FOR INFORMATION 785-296-1620	59	Serology	CDC
Poliovirus culture	Throat swab; feces (5 g); CSF (1-2 ml)	66	Viral Culture	
Protozoa, intestinal	SEE INTESTINAL PARASITES	42	Parasite	
Psittacosis serology (See Chlamydia Serology)	Acute and convalescent sera (3-5 ml each)	59	Serology	CDC
Q Fever serology	Acute and convalescent sera (3-5 ml each)	59	Serology	CDC

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Rabies, Direct Examination	Call Bureau of Epidemiology and Disease Prevention; Animal head or brain SEE APPENDIX C 877-427-7317	82 (RFFIT)	NA	KSU-CVM
Rabies, serology1	Serum (3-5 ml) SEE TECH. INFORMATION	82	Serology	KSU-CVM
Respiratory Syncytial Virus (RSV) culture2	Throat swab; nasopharyngeal aspirate	66	Viral Culture	
Respiratory Syncytial Virus (RSV) serology	Not available		NA	
Rickettsia serology SEE TECH. INFO.	Acute and convalescent sera 3-5 ml of each	53	Serology	
Ringworm	Not performed		NA	
Rocky Mountain Spotted Fever (RMSF)	Acute and convalescent sera 3-5 ml each for IgG-IFA. For IgM IFA assay call 785-296-1620	53	Serology	
Roundworms, intestinal (Ascaris lumbricoides)	SEE INTESTINAL PARASITES, Send adult worm in normal saline	42	Parasite	
Rubella, serology--Diagnosis (SEE TECH. INFO.)	Acute and convalescent sera 3-5 ml of each. For IgM assay call BEDP at 877-427-7317	56	Serology	
Rubella, serology--immune status (SEE TECH. INFO.)	Clotted blood or serum (3-5 ml)	56	Serology	
Rubeola, culture	Throat swab or washing, nasopharyngeal swab	66	Viral Culture	
Rubeola, serology (SEE TECH. INFO.)	Acute and convalescent sera 3-5 ml of each. For IgM assay call 785-296-1620	53	Serology	
St. Louis Encephalitis (SLE) serology	SEE ENCEPHALITIS, VIRAL SEROLOGY (SLE virus serology with WNV serology)	53	Serology	
Salmonella culture and/or Serotyping	Feces (marble-sized) in Cary-Blair or culture isolate	39	Enteric	
Salmonella serology	Serum (3-5 ml)	59	Serology	CDC
Scarlet Fever	SEE STREPTOCOCCUS	39	IDS	
Shigella culture and/or serotyping	Feces (marble-sized) in Cary-Blair or culture isolate	33	IDS	

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Schistosoma species	SEE INTESTINAL PARASITES	42	Parasite	
Schistosomiasis serology	Serum (3-5 ml)	59	Serology	CDC
Staphylococcus	SEE TECHNICAL INFO.	39	IDS	
Stool culture	SEE ENTERIC CULTURE	33	Enteric	
Streptococcus culture and/or grouping (SEE TECH. INFO.)	Culture isolate DM-7	39	IDS	
Strongyloides stercoralis	SEE INTESTINAL PARASITES	42	Parasite	
Strongyloidiasis serology	Serum (3-5 ml)	59	Serology	CDC
Syphilis serology, RPR (Serum), FTA-ABS-DS (Serum or CSF)	Serum or clotted blood (3-5 ml); CFS (2-3 ml)	63	Serology	
Tapeworms Dipylidium caninum, D. latum, Hymenolepis diminuta, H. nana, Taenia (speciated only with gravid proglottid)	SEE INTESTINAL PARASITES Send proglottids in saline	42	Parasite	
TB culture and/or smear	SEE TUBERCULOSIS	45	TB	
Tetanus	TELEPHONE EPIDEMIOLOGIC SERVICES 877-427-7317		IDS	
Toxocariasis serology (Toxocara canis)	Serum (3-5 ml)	59	Serology	CDC
Toxoplasmosis serology	Serum (3-5 ml)	59	Serology	CDC
Trichinella spiralis (Trichinosis) serology	Serum (3-5 ml)	59	Serology	CDC
Trichomonas hominis	SEE INTESTINAL PARASITES	42	Parasite	
Trichuris trichiura (Whipworm)	SEE INTESTINAL PARASITES	42	Parasite	
Trypanosoma species	SEE CHAGAS DISEASE, TELEPHONE FOR INFORMATION ON SPECIATION DM-11	43	NA	

Test	Specimen Required	Technical Information Page No. (Assay)	Shipping Kit Needed	Ref. Lab Used
Tuberculosis: smear, culture, drug susceptibility	Sputum, gastric wash, urine, etc. or culture isolate	45	IDS	
Tularemia	Culture isolate	39	IDS	
Tularemia serology	Serum (3-5 ml)	59	Serology	
Typhoid culture	SEE SALMONELLA CULTURE	34	IDS	
Vaccines	TELEPHONE FOR INFORMATION - DISEASE PREVENTION AND HEALTH PROMOTION (785) 296-5593			
Varicella (VZV) culture	Vesicle scrapings, SEE TECH. INFORMATION	66	Viral Culture	
Varicella (VZV) serology	Acute and convalescent sera (3-5 ml each)	53	Serology	
RPR (Non-treponemal Syphilis test)	Whole blood or serum (3-5 ml)	63	Serology	
Vibrio culture	SEE ENTERIC CULTURE	33	IDS	
Viral Culture	Viral transport media Ship cold (40 C)	66	Viral Culture	
Visceral larva migrans serology	SEE TOXOCARIASIS SEROLOGY	59	Serology	CDC
West Nile Virus serology	Call Epidemiological Services 877-427-7317 (CSF > 1 ml, serum 3-5 ml)	53	Serology	
Whipworms	SEE INTESTINAL PARASITES	42	Parasite	
Whooping cough	SEE BORDETELLA PERTUSSIS	40	PCR	
Worms, adult	Send worms in saline	42	NA	
Yeast culture	Not performed	47	NA	
Yersinia pestis (plague)	Culture isolate	39	IDS	
Yersinia enterocolitica	SEE ENTERIC CULTURE	33	IDS	

III. LABORATORY SPECIMEN KITS

LABORATORY SPECIMEN KITS

Laboratory specimen kits can be obtained by using the designated order form. A copy of this form can be downloaded from the laboratory web page at

www.kdhe.state.ks.us/sdm/download/specimen_kit_request_form.pdf

Please do not make requests on other laboratory forms or by sending notes. Orders for kits may be sent by FAX to the laboratory mail room at (785) 296-1641. Information about specimen kits, etc. can be obtained by calling the laboratory call center at (785) 296-1620.

Please restrict the number or kits requested to a quantity sufficient for one to two months of operation. If the normal usage rate is less than five kits per month then ordering a three month supply is recommended.

By requesting and using specimen kits and supplies from the Division of Health and Environmental Laboratories, you take the responsibility to follow these guidelines:

1. Division of Health and Environmental Laboratories kits and supplies are intended for collection and transport of specimen to the Division of Health and Environmental Laboratories only.

2. Use kits as designed; do not mix or delete components.

3. Always tape (using electrical tape or equivalent) any primary specimen tube with a screw-cap lid before sending kits to the Division of Health and Environmental Laboratories.

4. Follow procedures for shipment of clinical specimens and etiologic infectious agents (see APPENDIX A).

5. Return outdated or unused kits to the Division of Health and Environmental Laboratories.

6. All packing and shipping regulations, for United States Postal Service and all other State and Federal rules, apply to packages sent through the DHEL contracted courier service. All liability is with the shipper.

LIST OF SPECIMEN KITS AVAILABLE

SHIPPING KITS	CONTENTS	LAB	REMARKS
BACTERIOLOGY CULTURE			
Enteric Kit	• Outer box with inner plastic bottle holding a sterile, screw-cap jar containing Cary-Blair medium with 0.16% agar	DM	• For routine isolation of Salmonella, Shigella, E. coli 0157, and Campylobacter • Not for parasite specimens • Use only if media is pink in color
Gonorrhea Kit	• Thayer-Martin plate, plastic bag and CO_2 tablets • GC culture specimen form, Styrofoam container, cardboard outer sleeve	DM	• Incubate plates overnight at 35 C if possible • Send directly to State Laboratory
Miscellaneous - Infectious Substance Kit (IDS Kit)	• UN 6.2 certified infectious substance shipper • 1 Requisition form	DM	• Required for culture isolate for identification (enteric and aerobic bacteria, mycobacterium) • Complies with regulations for transport of microbial cultures
TUBERCULOSIS CULTURE			
Sputum Kit	• Outer box with inner plastic bottle containing 1 sterile 50 ml tube	DM	• Three early morning specimens on consecutive days • Do not send glass slides for smear exam
PARASITOLOGY			
Pinworms	• Outer mailing container • Pinworm collection tube • 1 Requisition form	DM	• Collect a morning specimen on three days • Available to Health Departments only
Parasite Stool	• Outer box with inner plastic bottle containing 1 bottle with PVA fixative solution and 1 bottle with 10% formalin • 1 Requisition form	DM	• For intestinal parasites, preservatives kill organisms. Unacceptable to culture
VIRAL & SEROLOGY			
Viral Transport Media	• 1 Sterile tube with 1 ml VTM • Return in Styrofoam box with cold gel pak and a Requisition form for each	VS	• **Health Departments only** * • Media not furnished for other Medical Facilities
Serology Kit for all blood, serum or CSF specimens submitted for a serology test at DHEL or CDC	• Outer box with inner plastic bottle containing a 5 tube Styrofoam insert • 1 Requisition form completed for each patient.	VS	• Collect at least 5 ml of whole blood or 3 ml of serum or 1 ml of CSF • Do not put more than 5 tubes into a shipping unit.
PCR			
For PCR assays	• Outer box • NP or Rectal Swab in Biohazard zip bag with the completed requisition form in the zip bags outer pouch placed in box (swab and zip bag not provided)	VS	• For Calicivirus PCR assays an Enteric Kit can be used. Send as soon as possible after collecting the specimens

SHIPPING KITS	CONTENTS	LAB	REMARKS
BLOOD LEAD			
Blood Lead Filter Paper Forms	• Blood spot collection form	IC	• Fill 3 circles with blood one at a time. Refer to specimen collection instructions
Blood Lead Venous Confirmation Kit	• Outer box and inner plastic bottle containing a 5 tube Styrofoam insert	IC	• Collect 3-5 ml of whole blood in EDTA (Purple top) • Do not put more than 5 tubes into a shipping unit.
NEONATAL SCREENING			
Genetic Metabolic Screening	• Blood spot collection form	NS	• Fill 5 circles with blood one at a time. Refer to specimen collection instructions

IV. NEONATAL SCREENING

TECHNICAL INFORMATION

METABOLIC DEFICIENCY DISORDERS

INTRODUCTION

Screening for neonatal phenylketonuria (PKU) was mandated in Kansas in 1965. The law was amended to include testing for congenital hypothyroidism in 1977, and amended in 1984 to add galactosemia screening. Screening for hemoglobinopathies commenced in 1990. Under State law, it is the responsibility of the person in charge of the hospital, birthing facility, or the attending physician to provide an appropriate blood specimen for PKU, hypothyroidism, galactosemia, and hemoglobin screening on all infants in their care. The specific details of newborn screening are addressed in Kansas Administrative Regulations for Phenylketonuria, Congenital Hypothyroidism, Galactosemia, and Sickle Cell Disease. A specimen for all newborns must be sent to the Kansas Department of Health and Environment. To ensure screening of all newborns in the State of Kansas, a blood specimen should be obtained prior to hospital discharge or prior to transfer to another hospital.

Neonatal screening has proven to be an effective method for identifying PKU, hypothyroidism, galactosemia, and hemoglobinopathies before mental retardation or morbidity occurs. Screening will identify infants in most cases before the onset of clinical symptoms. The Neonatal Screening Laboratory identifies newborns at risk for the above genetic and metabolic disorders. A definite diagnosis and therapy is provided either by the family physician or consultants to the newborn screening program.

Phenylketonuria (PKU) is an autosomal recessive inherited metabolic disorder due to an alteration or absence of the liver enzyme **phenylalanine hydroxylase**. Phenylalanine hydroxylase is necessary to convert (catabolize) the amino acid phenylalanine to tyrosine. In the absence of this enzyme, phenylpyruvic acid and other metabolites produced via alternate pathways are elevated in the blood. Neurological damage to the central nervous system results in mental retardation. There is not a consensus on exactly how retardation develops. The fair complexion and the blond hair are probably due to the inhibition of the enzyme (tyrosinase) which is necessary to produce melanin (pigments that give color to skin and hair).

An infant will appear to be normal at birth and during the neonatal period (first 28 days after birth). Evidence of mental retardation will normally be firmly established by 4 months of age. The disease affects one in 15,000 newborns. Treatment consists of a diet low in phenylalanine which must be started early and continued to prevent mental retardation and other complications. Treatment must include an educational component to prepare affected infants and parents to effectively manage the dietary restrictions and maintain psychological stability.

Congenital hypothyroidism is caused by a missing or inoperative thyroid gland. The primary function of the thyroid gland is the production of thyroid hormones (T4 and T3). The level of T4 and T3 in the blood is regulated by the thyroid stimulating hormone (TSH) produced in the anterior pituitary gland at the base of the brain. Under normal

physiological conditions a rising or elevated thyroid hormone (T4 and T3) level will inhibit the production of TSH through a feedback mechanism. In primary hypothyroidism the thyroid gland is unable to produce T4 and T3; therefore, the TSH level will continue to rise. Thyroid hormone (T4 and T3) increases the metabolic process of the body by increasing the consumption of oxygen, this is essential for normal brain development and growth. Congenital hypothyroidism affects approximately one in every 5,000 live births. Symptoms may occur within a week of birth or be delayed for months depending on the severity of the hormone deficiency. Treatment involves a replacement medication (thyroxine) for the lifetime of the affected individual. Screening of neonates expedites the diagnosis and early initiation of effective treatment to prevent a severe form of mental retardation.

Galactosemia is an autosomal recessive disorder of carbohydrate metabolism in which galactose cannot be converted to glucose because of defective or partially defective enzymes located in the liver, leukocytes, and red blood cells. Two different enzymatic defects can cause galactosemia, a deficiency of the enzyme **galactokinase** (less common and less severe) and **galactose-1-phosphate uridyl transferase** (severe type). The transferase enzyme can be either totally or partially defective. Classical galactosemia occurs when the transferase enzyme is totally defective. When the transferase enzyme is unable to convert galactose (galactose-1-phosphate) to glucose (glucose-1-phosphate), the liver and kidneys are injured by the toxic effects of galactose-1-phosphate. Cataracts are thought to be due to galactitol (dulcitol); therefore, it is seen with either of the enzymatic defect.

Partial enzyme activity can be detected on screening but requires additional testing to rule out activity lost due to non-genetic causes such as heat and improper collection techniques.

Symptoms may appear soon after the commencing of milk feeding or can be delayed until late infancy. A significant degree of mental retardation begins to occur about 30 days after birth. Treatment consists of removing galactose from the diet. The incidence of galactosemia is about one in 60,000 live births.

Hemoglobinopathies result from a defect in the gene producing hemoglobin. Hemoglobin S,C,D, and E are readily detected by the Newborn Screening Laboratory. The above
abnormal hemoglobin's result from the substitution of one amino acid in the red blood cell beta chain. Sickle cell disease results when both gene coding for red blood cells beta chain substitute the amino acid, valine, for glutamic acid. Hemoglobin SS (sickle cell disease) resulting from the substitution is likely to sickle in low oxygen tension environments such as joints and extremities; therefore, blocking the small blood vessels, which leads to ischemia and painful crisis. Hemoglobin C,D,E, and G patients usually present with some form of anemia when the condition is homozygous. Sickle cell disease occurs in 1 of 400 Afro-Americans, but is also seen in persons of Mediterranean, Asian, Caribbean, South and Central American ancestry. Screening for hemoglobinopathies will detect sickle cell disease and those newborns susceptible to

anemias in infancy. Infants born with sickle cell disease (hemoglobin SS) will be clinically normal. Infants with sickle cell disease have an increased risk of morbidity and mortality. From the age of about four months to three years, an infant with sickle cell disease is susceptible to septic infection (Streptococcus pneumoniae) and splenic sequestration (pooling of blood in the spleen) which are medical emergencies. The NIH Consensus Development Conference for Sickle Cell Disease and Other Hemoglobinopathies of April, 1987, recommended screening for sickle cell disease and prophylactic penicillin for infants with the disease.

SPECIMEN COLLECTION

A. Time of collection should be prior to hospital discharge. Infants who remain hospitalized for extended care should be tested at three days of age. Many infants are discharged from the hospital before two days of age. It is ESSENTIAL that the first specimen be collected just prior to discharge regardless of age. Infants screened prior to 24 hours of age should be recollected for repeat testing during the first two weeks of life. If an infant is transferred to another hospital, arrangements should be made to ensure that an initial specimen is obtained.

B. Blood should be obtained from the heel of the infant.

1. After the puncture site has been prepared, puncture the skin with a sterile disposable lancet.

2. Wipe away the first drop of blood with a sterile gauze pad, and allow a drop that is 1/6 to 1/3 the diameter of the printed circle to form. Touch the filter paper to the blood and fill the circle. Observe for complete saturation as the blood flows through the filter paper. Fill each circle in a similar manner. Do not over saturate.

3. Air dry the specimen at room temperature in a horizontal position for at least two hours before placing in an envelope for mailing.

4. Avoid touching the areas inside the printed circles before, during, and after collection.

5. CAREFUL INSPECTION TO ENSURE GOOD QUALITY BLOOD SPOTS AT THE TIME OF COLLECTION WILL SAVE VALUABLE TIME IN IDENTIFYING AFFECTED INFANTS.

C. Unreliable results may occur on the following specimens:

1. Specimens collected prior to 24 hours of age.
2. Specimens from infants of low birth weight.
3. Specimens from sick infants, or from infants who have been transfused.
4. Specimens that were collected into EDTA or Citrate tubes.

IDENTIFICATION AND SHIPMENT OF SPECIMENS

All information requested on the blood collection form is important. The collection form (kit) has a pamphlet attached for parental education. This Pamphlet comes in ENGLISH and SPANISH. If the Spanish version pamphlet is not requested the English version pamphlet will be sent.

A. Please do not write in the lab number space at the top of the form.

B. For first-time specimens, please use the collection kit labeled "FOR INITIAL SCREENING ONLY."

C. When a repeat specimen is submitted, please use the collection kit labeled "FOR REPEAT SCREENING ONLY."

D. **Please mail specimens within <u>24 hours</u> after collection**.

E. The laboratory will test all specimens with an adequate amount of blood on the collection filter paper. Specimens not meeting all criteria for a satisfactory sample will be tested. A laboratory report will be sent to the submitter bearing an **<u>UNSATISFACTORY</u>** notice and an explanation for the reason(s) for rejection. A second specimen should be obtained as soon as possible.

F. If less than 24 hours have elapsed between time of birth and time of specimen collection, there is a risk (approximately 16% in the first 24 hours at a 4 mg% cutoff and 2% in the second 24 hours of life) that the PKU test results are not valid due to an inadequate phenylalanine intake (Committee on Genetics, American Academy of Pediatrics, Pediatrics, January 1982, p. 104). The PKU test is performed on these specimens, but the laboratory report will bear an advisory note. Because of the possibility of a false-negative test result, it is recommended that the physician send a repeat specimen during the first two weeks of life.

INTERPRETIVE DATA

All follow-up activities are conducted by the Follow-up Program component of the Newborn Screening System (The Children's Developmental Services section in the Bureau of Children, Youth, and Families provides additional information with reports to physicians and assists with referral for positive congenital hypothyroidism screening results (785-291-3363)

.

The following information is used to implement follow-up procedures for presumptive positives. These values are not meant to be used as diagnostic tools, but rather to

provide physicians with some guidelines for the interpretation of Neonatal Screening results:

PKU
Negative = Less than 2.1 mg%
Equivocal = 2.1-2.9 mg%
Positive = Greater than or equal to 3.0 mg%

Congenital Hypothyroidism
All infants receive a TSH test. A presumptive positive has a Thyroxine (T4) value in the lowest 25% of a daily batch with a Thyroid Stimulating Hormone (TSH) value greater than or equal to 20 µIU/ml. A specimen with a TSH value greater than or equal to 60 µIU/ml is considered a presumptive positive regardless of the T4 value. In addition, the highest 1% of the remaining TSH values, 20 µIU/ml or above, not already considered a presumptive positive will also be reported as presumptive positives. Using the above criteria, some infants with TSH values greater than 20 µIU/ml will be negative for primary hypothyroidism.

Galactosemia
Negative = Normal screening result
Positive = Presumptive positive for galactosemia

(Galactosemia screening tests are qualitative; no values are reported).

The above program notifies physicians of all presumptive positive screening results.

For information concerning newborn screening regulations, follow-up recommendations, or a list of clinical consultants, please call (785) 291-3363.

For technical information concerning specimen collection or screening tests, please call (785) 296-1620.

REFERENCES

KSA 65-180 through 65-183; Kansas law concerning neonatal screening for phenylketonuria, congenital hypothyroidism, and galactosemia.

KAR 28-4-501 through 28-4-513; Kansas Administrative Regulations for Phenylketonuria, Congenital Hypothyroidism, and Galactosemia.

Neonatal Screening for Inborn Errors of Metabolism. H. Bickel, R. Guthrie and G. Hammerson (eds.) Springer-Verlag; Berlin, Heidelberg, New York, 1980

NIH Sickle Cell Disease Consensus Conference. April 6-8,1987, vol 6(9)
Committee on Genetics, American Academy of Pediatrics, Pediatrics, Jan. 1982, p. 104

V. MICROBIOLOGY

TECHNICAL INFORMATION

MICROBIOLOGY

INTRODUCTION

Microbiology is composed of two major laboratory units: The Bacteriology/Parasitology Unit and the Mycobacteriology Unit.

Services in the Bacteriology/Parasitology unit include enteric bacteriology, food bacteriology, culture diagnosis of *N. gonorrhoeae*, reference identification of bacteria, and parasitology.

The Mycobacteriology unit provides culture and identification for all mycobacteria and drug susceptibility testing for *Mycobacterium tuberculosis*.

ENTERIC BACTERIOLOGY

INTRODUCTION

The Enteric Laboratory examines fecal specimens for enteric pathogens and identifies reference cultures belonging to the family *Enterobacteriaceae*.

Specimens submitted for enteric culture are routinely examined for the presence of *Salmonella*, *Shigella*, *E. coli* 0157:H7, and *Campylobacter* species. In addition to identifying these common enteric pathogens, the Enteric Laboratory will also isolate and identify Vibrio species and *Yersinia enterocolitica* on request. Drug susceptibility testing is available for *Shigella* and *E. coli* isolates. Requests for *Salmonella* susceptibility results require approval by the Microbiology section at 785-296-1620.

Serotyping of *Salmonella* and *Shigella* species is performed on all isolates. Note that state regulations require that all isolates of *Salmonella*, *Shigella*, and *E. coli* 0157:H7 be sent to the state laboratory. Requests for serotyping of potentially enteropathogenic strains of *Escherichia coli*, other than *E. coli* 0157:H7, should be related to an outbreak of *E. coli* and requires approval of the Microbiology section at (785) 296-1620. Testing of *E. coli* isolates for Shiga toxins I and II by ELISA and PCR is available and positive isolates will be sent to CDC for serotyping.

SPECIMEN COLLECTION

The primary specimen container must be labeled with the patient's name or other unique identifier. Unlabeled specimens will be rejected. A request form must accompany the specimen(s) and should be filled out completely. Please return expired kits to the state laboratory and do not use them beyond the expiration date on the mailer.

A. Feces Specimen: Stool specimen kits supplied by this laboratory include a specimen bottle containing a pink to red colored Cary-Blair medium (0.16% agar concentration). This medium is designed for the transport of stool specimens for the recovery of enteric pathogens. If the contents of the specimen bottle are not pink to red in color, do not use. Return the kit and request a replacement.

Stool specimens should not be mixed with foreign material such as water from the toilet or with urine. The feces can be passed onto dry surface such as newspaper, bedpan, or plastic wrap. From this, collect a marble-sized mass of feces using applicator sticks (not provided) and place this amount into the specimen bottle. Mix thoroughly by shaking the bottle vigorously after the bottle cap has been tightened securely.

Alternately, a rectal swab sample can be obtained by pushing a swab gently into the rectum with careful avoidance of skin contact of the perianal area. Immerse the swab in the transport medium of the specimen bottle and break off the swab shaft below the cap level of the bottle. Tighten cap, shake bottle vigorously, and mail to the laboratory for examination.

B. Culture Isolates: Submit a pure culture on a nutrient agar slant or similar medium that does not contain carbohydrates. Note that state regulations require that all isolates of *Salmonella*, *Shigella*, *E. coli* 0157:H7, *Neisseria meningitidis*, and invasive group A streptococci or invasive *Streptococcus pneumoniae* must to be sent to the state laboratory.

SHIPMENT OF SPECIMENS

Kits for submitting fecal specimens and mailing containers for submitting reference cultures are available upon request (see p. LS-2, LIST OF SPECIMEN KITS AVAILABLE). **Note: culture isolates are classified as dangerous goods and must be transported in a UN 6.2 certified infectious substance mailer.** The infectious substance mailer is available from the laboratory mail room (785-296-1620).

Once fecal specimens are placed in the Cary-Blair medium, the specimen should be refrigerated until transported to the Division of Health and Environmental Laboratories. Specimen shipment must be made in such a manner that transit time is minimized. In the case of an enteric disease outbreak contact the Epidemiologic Services at 877-427-7317 to determine the number of specimens to be collected. Please advise the laboratory by telephone so that supplies may be prepared.

REPORTING PROCEDURES

Negative culture reports will indicate "*Salmonella*, *Shigella*, *Campylobacter*, and *E. coli* 0157:H7 Not Found." Positive cultures will specify which organisms were found and for *Salmonella* the serotype will also be reported. In the case of special requests the report will also indicate whether or not the requested organism was found. The laboratory will

provide upon request a record of the tests performed to establish the definitive identification of the organism.

Clinically relevant culture results are telephoned as soon as available and followed by a written report.

REFERENCES

Murray, P. R. et. al. (Ed.) 2003. *Manual of Clinical Microbiology*, 8th ed. American Society for Microbiology, Washington, D.C. 20005

FOOD BACTERIOLOGY

INTRODUCTION

Microbiological analysis of food is provided in support of an investigation into a food borne disease outbreak and is limited to testing for those organisms which have been implicated in the outbreak.

SUBMISSION OF FOOD SAMPLES FOR ANALYSIS

All requests for laboratory examination of food or food-related samples must be made through Epidemiologic Services at (toll free) 877-427-7317. Laboratory examination of food will be performed only when approved by these units. Results of food analysis will be reported to the Epidemiologic Services section and the local health department to assist them in an outbreak investigation.

The value of laboratory results in microbiology is directly dependent on the quality of samples submitted. Food samples must be obtained using aseptic technique and appropriate containers. Samples must be refrigerated during storage and transport, and must arrive at the food microbiology laboratory within three days of collection. Samples collected frozen should be stored and transported frozen (on dry ice).

Collect samples:
1. Use sterile containers and ensure that leakage is prevented.
2. Wash hands prior to putting gloves on.
3. Do not touch inside of collection container.
4. Use sterile utensils, such as a tongue depressor to collect food.
5. Collect an adequate amount of the sample—a minimum of 4-6 ounces, if possible.
6. Fill the container no more than ¾ full.
7. Keep food cold by placing it in the styrofoam cooler with an ice pack. Use additional ice as necessary.
8. Clearly document how the product was handled and who handled it after the sample is taken.

Label food samples with name and type of product, brand of product, product manufacturer, inspector name, date, time, and place of collection, and establishment name

Complete the laboratory requisition form. On Page 1, under “Sample Information” and subheading “Clinical Source”, mark “Other” and write ‘Food Sample’. On Page 2, under the subheading “Submitter Comments”, indicate that the food is related to an outbreak investigation and that testing was approved by KDHE Epidemiology at (877) 427-7318. The sample should be sent via the KDHE courier or FedEx.

CULTURE DIAGNOSIS OF N. GONORRHOEAE
Non-Genito-Urinary Tract (GU) and Medical-Legal Specimens

INTRODUCTION

Routine testing of GU specimens for the presence of *N. gonorrhoeae* is performed using nucleic acid amplification technology. These tests are performed by the Virology/Serology laboratory, refer to the Virology/Serology section of this manual for information on routine testing or call 785-296-1620. At this time amplified nucleic acid testing is approved ONLY for genitourinary tract specimens and urine specimens and cannot be used for specimens from other body sites or those intended for medico-legal purposes. Tests on specimens from other sites (joint aspirates, throat, eye, rectum, etc.) or for medico-legal purposes must be analyzed by the culture method described below.

SPECIMEN COLLECTION

Specimens must be inoculated immediately onto modified Thayer Martin agar plates which are available from this laboratory. The plate is inoculated by rolling the specimen swab across the medium surface in a large "Z" pattern to assure thorough contact of the swab with the medium. After rolling, cross streak the "Z" with the tip of the swab in overlapping strokes over the entire agar surface. After the agar plate has been inoculated do the following:

- Write patient name and source of specimen (rectal, throat, etc.) on exterior surface of plate with a marking pen.
- Place the plate inside the plastic bag provided with the kit.
- Open one side of the foil packet containing the CO_2 tablet and put entire packet inside the bag along with the agar plate. DO NOT REMOVE CO2 TABLET FROM FOIL PACKET. DO NOT PUT CO_2 TABLET INSIDE THE AGAR PLATE.
- Squeeze excess air from bag then seal the bag by holding the wire tabs and whirling the plate in the bag around them and then bending the tabs back along the wire.
- Complete the laboratory request form by filling in all the information requested.
- *N. gonorrhoeae* is more readily recovered if the plate is incubated 16 to 18 hours at 35°C before shipping. However, if incubation is not possible, hold the bagged plate at room temperature until the specimen is sent. **DO NOT REFRIGERATE**.
- Send the specimen to the laboratory as quickly as possible, using the laboratory styrofoam shipping system.

SHIPMENT OF SPECIMENS

Gonorrhea culture plates should be sent to the laboratory after inoculation and/or preliminary incubation. Shorter transit time may improve recovery of *N. gonorrhoeae*. Use only the shipping containers provided by the Division of Health and Environmental

Laboratories for shipment of these specimens as these provide the most protection during transit and facilitates sorting on receipt. Any exceptions to use of the State mailer kit must be approved by the Diagnostic Microbiology staff (785-296-1620).

LABORATORY PROCESSING

In the laboratory, identification is based on:

- Gram stain showing gram negative diplococci with typical colony formation on culture plates.
- Positive oxidase reaction.
- Positive DNA Genetic Probe Result
- Additional biochemical confirmation results as indicated.

REPORTING PROCEDURES

Cultures where *Neisseria gonorrhoeae* could not be found are reported as "*Neisseria gonorrhoeae* NOT FOUND." Cultures positive for *Neisseria gonorrhoeae* are reported as "*Neisseria gonorrhoeae* FOUND."

Unidentified specimens and specimens without a provider's address are discarded and reported as "Unsatisfactory--Specimen not identified" or "Unsatisfactory--Provider's address not given." Specimens that have an excess amount of fluid in bag are discarded, and are reported as "Unsatisfactory--excess fluid in bag."

Negative specimens that are overgrown with contaminants, that are too long in transit (> 72 hours), that are inoculated on expired media, or that are received in an inappropriate condition are reported as "*Neisseria gonorrhoeae* not found," with a note explaining why the test may have been compromised. Results of "*Neisseria gonorrhoeae* found" are telephoned to those health care providers requesting telephone reporting. Written reports are mailed for all specimens received.

REFERENCES

Kellogg, Douglas S., Jr., et al. Oct. 1976. “Laboratory Diagnosis of Gonorrhea”, In *Cumitech 4*. Washington, D.C.: American Society for Microbiology.

Murray, P. R. et. al. (Ed.) 2003. *Manual of Clinical Microbiology*, 8th ed. American Society for Microbiology, Washington, D.C. 20005.

Centers for Disease Control. 1976. *Procedures for Use by the Laboratory in the Isolation and Identification of Neisseria gonorrhoeae*.

REFERENCE BACTERIOLOGY

INTRODUCTION

The primary function of the Reference Bacteriology Laboratory is to assist Kansas clinical laboratories and physicians in the identification, confirmation, and characterization of bacterial isolates of medical or bioterrorism significance. Emphasis is placed on the identification of unusual isolates, potential bioterrorism agents, and other organisms which are not readily identified by automated microbiology systems.

REFERENCE BACTERIOLOGY LABORATORY TESTS

- Biochemical identification of nonfermenters, non-enteric fermenters *Listeria*, etc.
- Bioterrorism-related organism identification including *Bacillus anthracis, Yersinia pestis, Francisella tularensis,* and Brucella species.
- Serotyping of *Neisseria meningitidis* and *Haemophilus influenzae* group b.
- Detection of *Bordetella pertussis* in nasopharyngeal specimens by PCR and identification of culture isolates by a direct fluorescence antibody test.
- Culture and identification for *Corynebacterium diphtheriae*.
- Lancefield grouping of beta hemolytic streptococci.
- Species determination of *Neisseria, Streptococcus,* and *Staphylococcus*.
- Legionella specimens are referred to CDC.
- Anaerobic identification is not performed but clinically relevant isolates can be sent to CDC for identification. Call the Bacteriology Laboratory before sending isolates.
- Staphylococcus DNA fingerprinting: available for epidemiologic investigations. When DNA fingerprinting is required call the Diagnostic Microbiology section at (785) 296-1620 for information and requirements.

Additional reference tests are available at CDC when referred through this laboratory; please contact the Bacteriology Laboratory for more information at (785) 296-1620.

GENERAL INSTRUCTIONS FOR SENDING REFERENCE CULTURES

Cultures should be clinically relevant, not contaminants or normal flora components. Only pure cultures should be submitted on agar slants with screw caps to prevent leakage in transit. **Note: culture isolates are classified as dangerous goods and must be transported in a UN 6.2 certified infectious substance mailer.** The infectious substance mailer is available from the laboratory mail room (785-296-1620). Complete a laboratory request form. Specimens must be identified by name or other unique identifier. It is helpful to know which bacterial agent is suspected when a culture is submitted. When specimens or cultures require referral to the CDC, Form 50.34-CDC Referral Form, will need to be completed by the originator of the specimen before the specimen can be sent to the CDC. A copy of the form is in Appendix A or forms can be obtained through our Sample and Data Management Office. For further information and additional forms, please call (785) 296-1620.

TYPES OF CLINICAL SPECIMENS

A. Nasopharyngeal swabs for Bordetella pertussis by PCR (Note: culture is provided only by prior arrangement, see note below) The specimen of choice is a nasopharyngeal swab collected pernasally: A commercial bacterial nasopharyngeal swab collection system should be used, such as the Bacti-Swab NPG Collection and Transport System available from REMEL as catalog # 12-300. (Note: citation of a product by name is not an endorsement by the state laboratory). With the patient's head tilted back about 70° from vertical and immobilized, gently insert the swab into a nostril until resistance is encountered as the swab contacts the nasopharynx. Patient tolerance permitting, try to leave the swab in this position for 10 seconds to absorb nasopharyngeal secretions. However, the tickling sensation of the swab usually induces a cough and good clinical judgment should dictate when to remove the swab.

Sample each nares with the swab and return the swab to the transport tube. **DO NOT** prepare smears from the swab.

Label the transport tube with the patient's name and attach a barcode from the form and complete the laboratory requisition including checking the pertussis PCR box on the back of the form. Send the completed laboratory request form and the swab to the Division of Health and Environmental Laboratories. The specimen can be shipped at room temperature since viable bacteria are not required for a PCR analysis.

NOTE: Analysis by PCR is the method of choice. The laboratory does not routinely provide media for Bordetella culture but can do so under special circumstances, usually in conjunction with an outbreak investigation by Epidemiologic Services. Experience suggests that when attempting culture isolation for *B. pertussis* that inoculation of either Regan-Lowe and/or Bordet-Gengou media immediately upon specimen collection is essential for recovery. The bacteria usually do not survive when transported before inoculation onto culture media in the state laboratory.

B. Blood, CSF, and Urine for *Leptospira*

During the first week of illness in humans, blood and CSF are specimens of choice for the culture of Leptospira sp. After the first week of illness, blood and CSF specimens rarely yield leptospires, however, at this time and for several months thereafter, the urine may contain low levels of intermittently shed leptospires.

Culture in appropriate media is important, however, the Diagnostic Microbiology Laboratory does not provide Leptospira culture media nor are there any

commercial media sources in Kansas. **Please call to make arrangements for specimens for culture to be sent to CDC.**

C. Throat swab for culture of Diphtheria.

Diphtheria may cause a peritonsillar pseudomembrane or throat inflammation. Collect either a swab of the membrane or inflamed area or a portion of the pseudomembrane. Transport in a sterile, dry container. Swabs should not be placed in transport media.

Submit each labeled specimen along with a completed requisition form to the Division of Health and Environmental Laboratories using overnight mail.

REPORTING

Results of biochemical or serological tests are returned with final culture reports. Most cultures are reported in five to seven days: mixed cultures and slow-growing organisms often require more time. Diphtheria toxin production is the definitive characteristic of pathogenic *C. diphtheriae*. Isolates will be sent to CDC to confirm the presence of diphtheria toxin. Significant results are telephoned prior to sending written results.

REFERENCES

Murray, P. R. et. al. (Ed.) 2003. *Manual of Clinical Microbiology,* 8th ed. American Society for Microbiology, Washington, D.C. 20005

PARASITOLOGY

INTRODUCTION

The Parasitology section detects and identifies intestinal parasites human illness. Specimens from non-intestinal sites are accepted for examination or referral when clinical symptoms suggest a parasitic involvement. Medically significant arthropods and other unusual specimens will also be accepted and referred for expert analysis as needed.

SPECIMEN COLLECTION

Each specimen must be labeled with the patient's name or other unique identifier. The request form accompanying the specimen must be filled out completely. Unlabeled specimens will be rejected and reported as unsatisfactory. Do not use kits beyond the expiration date on the mailer.

A. Intestinal Parasites: The laboratory provides the traditional two vial intestinal parasite collection kit. Commercially available single vial collection kits are accepted but these may not preserve the parasites as well as the two vial systems. Since some parasites are passed intermittently collection of specimens on at least two consecutive days is recommended to improve detection. Collect one specimen per day until two to three specimens have been collected.

- Fecal Specimens: Stool specimens should not be mixed with foreign material such as water from the toilet or with urine. The feces can be passed onto dry surface such as newspaper, bedpan, or plastic wrap. From this, collect a marble-sized mass of feces using applicator sticks (not provided) and place this amount into the specimen bottle. Mix thoroughly by shaking the bottle vigorously after the bottle cap has been tightened securely. Try to minimize stool contact with water, dirt, or urine, as these contaminants can lead to degeneration of the parasites and a missed diagnosis. Specimens containing anti-diarrheal compounds, barium, bismuth, or mineral oil interfere with the parasite examination and render the specimen unsatisfactory for diagnosis. Note that antibiotic therapy can cause a temporary decrease or absence of organisms for two or three weeks. Both vials (formalin and PVA) are essential for an accurate examination. The fecal material should be put in the preservatives as soon as it is passed. Thorough mixing of the specimen with the preservatives is important. Do not refrigerate the preservative before or after adding the specimen. The PVA preservative congeals at refrigerator temperatures and the specimen is unusable. If the PVA vial in the kit is congealed when received, as can happen in the winter, it may turn back to liquid if heated in hot tap water (50°C).

- Pinworm Specimens: This test is provided to county health departments only. Collect specimens by pressing the adhesive paddle on and around the rectal

mucosa to pick up the Pinworm eggs. Collect the specimen first thing in the morning before the eggs are dislodged. Place the paddle back in its tube and put the tube in the bottle to return to the laboratory. Collection on three consecutive days is recommended. Try to avoid getting fecal material on the slide as this obscures the pinworm ova and makes it hard to see them during microscopic examination.

- Whole Worms or proglottids: Place the specimen in a clean, screw-capped container with physiological saline and mail to the parasitology laboratory immediately. Please **DO NOT** use a bottle containing preservative because this will interfere with the staining used to highlight the internal structures.

B. Other Specimens:

- Cryptosporidium: Available on request for patients meeting at least one of the following criteria: watery diarrhea, immunosuppressed, less than 5 years of age, institutionalized, or the contact of a known case.

- Urine (Submit Only Formalin Preserved Urine Specimens): The optimal urine specimen for revealing eggs of *Schistosoma haematobium* has been shown to be one passed about or shortly after noon. Eggs are more frequently present in the last few drops of the specimen. Exercise also increases the chances of finding eggs. Daily examinations for three consecutive days should be performed. It is essential that a request for this test be noted on the requisition form accompanying a urine specimen.

- Sputum: In addition to feces, sputa can be collected in suspected cases of paragonimiasis. Formalin preserved sputum is preferred.

- Duodenal Drainage Material: For infections with *Strongyloides stercoralis* and *Giardia lamblia*, duodenal drainage often reveals organisms when stool specimens are negative. Formalin-preserved specimens are preferred.

- Sigmoidoscopic Material: Formalin-preserved material is preferred.

- Aspirated Material: Liver abscesses are commonly aspirated for parasitic examination. Generally, organisms are located in the peripheral area of a liver abscess. At least two portions of the exudate should be removed. The first portion, usually yellowish-white, seldom contains amoebae. Later portions, reddish in color, are more likely to contain organisms, and the portion containing material from near the wall is most likely to be positive for *Entamoeba histolytica*. Phone inquiries are encouraged when questions arise regarding parasitic examination of any unusual specimen. Formalin and PVA preserved material is preferred.

C. Arthropods: Ticks and other ectoparasites can be submitted for identification. Unusual organisms are identified by arrangement with the entomology department at KSU. Preservatives and transport conditions vary with the type of insect, please call the lab at (785) 296-1620 for handling and shipping information.

D. Blood and Tissue Parasites: Certain parasites will be found in the peripheral blood in the highest numbers at different times. In the case of suspected filariasis, the laboratory should be contacted for the optimum collection times.

Blood smears for Malaria, *Babesia*, and other blood parasites can be made with blood from either finger prick or venipuncture. Blood films should be prepared before anticoagulants are added because the addition of these will prevent blood from sticking to the slide and may interfere with staining. Prepare a thick and thin smear (call for instructions, if needed). Do not use a fixative. If possible also send a whole blood specimen in EDTA anti-coagulant (purple top tube). Parasites in blood smears will be identified by CDC.

SHIPMENT OF SPECIMENS

Ship all specimens as quickly as possible after collection. Choose collection dates so that there will be limited delay in transit time to the laboratory and try to avoid collection times which will be delayed over the weekend before specimen arrives at the laboratory. Please contact the laboratory before sending unusual or large numbers of specimens (epidemics or surveys) to establish that the specimens can be analyzed.

REPORTING PROCEDURES

Results are generally reported in two or three days following the arrival of the specimen. More time may be required in unusual situations. Results will be telephoned upon request.

REFERENCES

Garcia, Lynne S. 1999. *Practical Guide to Diagnostic Parasitology.* American Society for Microbiology, Washington, D.C. 20005

Murray, P. R. et. al. (Ed.) 2003. *Manual of Clinical Microbiology*, 8th ed. American Society for Microbiology, Washington, D.C. 20005

Garcia, Lynne S. and David A. Bruckner. 1993. *Diagnostic Medical Parasitology*, 2nd ed. American Society for Microbiology, Washington, D.C. 20005

MYCOBACTERIOLOGY

INTRODUCTION

The Mycobacteriology Laboratory provides isolation and identification of Mycobacteria from clinical specimens and identification of cultures referred from any health care provider for a Kansas resident. Culture and identification are performed using current rapid methods such as broth culture, nucleic acid amplification and probes, and high performance liquid chromatography (HPLC). Specimen smears are read using a sensitive fluorochrome method and reported within 24 hours of specimen receipt except during weekends and holidays. Direct specimen amplified nucleic acid testing is performed within one working day of detecting a positive smear on respiratory specimens. Susceptibility testing is performed on both clinical and reference isolates of *M. tuberculosis* and *M. bovis*.

The primary mission of the unit is to support the Tuberculosis Control Program of Kansas and local agencies in the control of tuberculosis. This includes referring TB isolates for DNA fingerprinting analysis.

SPECIMEN COLLECTION

Each specimen/culture must be identified with either the patient's name or other unique identifier. A completed request form must accompany the specimen. Unlabeled specimens will not be processed.

A TB mailer with a sterile container is available at no charge from the Division of Health and Environmental Laboratories mailroom. Collecting specimens:

A. Sputum. Sputum specimens should be collected in the early morning. A series of three separate specimens taken on consecutive days is best for detecting mycobacteria. When patients are unable to produce sputum, specimens can be induced through inhalation of a warm sterile mist of 10% NaCl. An optimum sputum specimen consists of 5 to 10 ml of material coughed from the bronchial tree (patients need to be instructed to collect sputum rather than saliva or nasopharyngeal exudates).

B. Gastric lavage: Specimens from patients who cannot produce sputum can be obtained by gastric lavage. Call the Mycobacteriology section at (785) 296-1620 for special instructions prior to collecting gastric specimens.

C. Urine: Three midstream specimens collected in the early morning on consecutive days are preferred. Twenty-four-hour pooled specimens are unsatisfactory due to contaminant overgrowth. Urine specimens may be transported using the TB kit. Specimens awaiting transport should be refrigerated.

D. Tissue: Tissue specimens must be collected aseptically and submitted in a sterile container. Use the TB kit provided by this laboratory for specimen submission.

E. Stool: Stool specimens can be of use in detecting and monitoring disseminated *Mycobacterium avium* complex infections in immunocompromised patients. A marble-sized volume is sufficient for testing. Note that high levels of endogenous flora may overgrow any mycobacteria present. Use the TB kit provided by this laboratory for specimen submission.

F. Miscellaneous Specimens: Pleural fluid exudates or aspirates from lesions, spinal fluid, bronchial washings, joint fluid, and laryngeal swabs should be collected aseptically and submitted in sterile containers. The volume of some of these specimens may be small and may require careful handling in order to prevent loss. Use the TB kit provided by this laboratory for specimen submission.

F. Reference Cultures: Inoculate a slant and incubate until good growth is established. DO NOT send cultures in petri dishes. Send on mycobacteria medium in a taped, screw-capped culture tube.

G. Prepared smears for AFB staining should not be mailed to the Mycobacteria Laboratory. Smears will be prepared from the clinical material submitted.

SHIPMENT OF SPECIMENS

Culture isolates are classified as infectious substances and must be transported in a UN 6.2 certified mailer. The infectious substance mailer is available from the Sample and Data Management section of the laboratory (785-296-1620).

REPORTING PROCEDURES

A. Microscopic examination results of clinical specimens are available within 24 hours of receipt except weekends. Results are reported as follows:

- No Acid-fast Bacilli Found
- 1+ Acid-fast Bacilli Found (= 4 - 40 / 100 high power fields)
- 2+ Acid-fast Bacilli Found (= 4 - 40 / 10 high power fields)
- 3+ Acid-fast Bacilli Found (= 4 - 40/ high power field)
- 4+ Acid-fast Bacilli Found (> 40 / high power field)

B. Growth of Mycobacteria

- Preliminary report by phone and mail as soon as acid-fast growth is detected
- Final report upon completion of identification

C. Identification of Mycobacteria

- All acid-fast culture growth is identified by HPLC, a rapid same-day procedure and the results are reported by telephone
- Some isolates which cannot be identified by HPLC are identified by slower traditional methods which may require up to 4 weeks

D. Susceptibility results are provided only for *M. tuberculosis* or *M. bovis,* cultures of other species of mycobacteria will be provided upon request for submission to private laboratories for drug testing. The following primary drugs are tested for TB/bovis, secondary drugs are available from CDC:

- Isoniazid
- Rifampin
- Pyrazinamide
- Ethambutol
- Streptomycin

E. Specimens will be reported as unsatisfactory if:

- Specimen not properly identified
- No specimen provided
- Provider's address not given

F. Clinically significant laboratory results (positive smear, culture growth, culture identification, and drug susceptibility results) are telephoned immediately and followed by a written report.

REFERENCES

Murray, P. R. et. al. (Ed.) 2003. *Manual of Clinical Microbiology*, 8th ed. American Society for Microbiology, Washington, D.C. 20005

Kent, Patricia T., and Kubica, George P. 1985. *Public Health Mycobacteriology: A Guide for the Level III Laboratory*. U.S. Public Health Service, Centers for Disease Control and Prevention, Atlanta, GA 30333.

MYCOLOGY

Mycology services are not offered by the laboratory, however, isolates of suspected systemic fungi such as *Blastomyces*, *Histoplasma*, *Sporothrix*, *Coccidioides*, etc. will be sent to CDC for identification/confirmation.

VI. VIROLOGY AND SEROLOGY

TECHNICAL INFORMATION

CHLAMYDIA DETECTION BY CULTURE METHOD OR BY A NUCLEIC ACID AMPLIFIED TEST (NAAT) FOR DETECTION OF *C. trachomatis* & *N. gonorrhoeae*

INTRODUCTION

Chlamydia trachomatis and *Neisseria gonorrhoeae* are major causes of sexually transmitted disease (STD) morbidity in the United States. The chlamydia are obligate intracellular parasites, classified as gram negative bacteria, but lacking in some important metabolic mechanism(s) found in free-living bacteria. *Neisseria* are also gram negative bacteria with similar pathology, however, they can be propagated in selective media as free-living bacteria.

For information concerning culturing of *N. gonorrhoeae* see page 37 of this Manual or call Diagnostic Microbiology Laboratory at 296-1620.

Diseases associated with these two organisms include inclusion conjunctivitis, neonatal pneumonia acquired at birth, trachomatis and most frequently urogenital tract infections including lymphogranuloma venereum (LGV), urethritis and cervicitis.

The chlamydia bacteria have been classified into three subgroups. STD associated infections are caused by *C. trachomatis* (Subgroup A), which can be propagated in McCoy cells, provided proper collection and rapid shipment of specimens in a chlamydia specific transport system (see APPENDIX D) is used.

Other chlamydial agents; *C. psittaci* (Subgroup B) is an animal pathogen with accidental infections in humans which is associated with Psittacosis-Ornithosis and meningo-pneumonitis, while Subgroup C contains *C. pneumoniae* formerly identified as the TWAR variant. This agent has been shown to be a significant respiratory pathogen. Please direct questions about *C. psittaci* and *C. pneumoniae* infections to the Virology Laboratory at 785-296-1620.

SPECIMEN COLLECTION AND IDENTIFICATION FOR CULTURE METHOD

Collection instructions for culture detection of *C. trachomatis* infections in humans are given below. Do not use Dacron swab for isolations.

A. Male genital: Urethral specimens should be obtained by inserting a small calcium alginate swab 3 to 6 cm into the urethra and firmly rotating it. The swab is removed and then broken off into 2SP transport medium or use a Chlamydia male collection swab culturette system.

B. Female urogenital: Using a calcium alginate swab, scrape the endourethra 4 to 6 cm from meatus. For a cervical culture, first wipe the transitional zone of the cervix with a swab and then use a second swab to obtain the specimen. This should be rotated to obtain cellular material. Break off the swab into 2SP transport medium or utilize a Chlamydia female collection swab culturette system.

C. Eye: A swab of conjunctival discharge is obtained by firmly stroking the palpebral conjunctiva. Break off swab into 2SP transport medium or use a Chlamydia specific swab culturette system.

D. Infant pneumonia: Nasopharyngeal aspirates should be transferred into a vial of 2SP transport media. If swabs are taken, they must be posterior of the nasopharynx since throat and oropharynx specimens are unsatisfactory.

E. LGV pus and aspirates: Aspirate pus from a fluctuant lymph node (bubo) and transfer aspirates into a vial of 2SP transport medium.

If possible, aseptically cut or break off the swab in collection tube, leaving the swab as long as practical. Close cap tightly and secure with tape. If using a Chlamydia collection system, make sure cap is tightly pushed onto the culturette holder.

Genital secretions and urine specimens are not adequate for Chlamydia cultures.

Clearly label each vial of 2SP transport medium or collection tube with patient's name, type of specimen, date of collection and bar code for Universal Requisition form used. Complete the form specifying Chlamydia culture with all requested data filled in on the front and back of the form.

Store 2SP (CTM) collection medium at 4°C for up to one year (Do Not Freeze).

SHIPMENT

Keep specimen cold (several ice packs) if delivered to the Virology Laboratory within 12 hours; otherwise freeze specimen and ship at -70°C (dry ice) within five days. When shipped on dry ice, seal specimen in a plastic bag to prevent absorption of CO2. See shipment instructions for "Virus Culture" on page 66.

REPORTING PROCEDURE

Positive cultures will be reported when cytoplasmic inclusion bodies are observed following immunofluorescent staining after 48 hours of growth in cycloheximide treated McCoy cells. Failure to detect inclusions at that time will result in a negative observation report; however, this does not rule out the possibility of Chlamydia infection. Turnaround time is from four to six days (excluding mailing time).

DUAL *Chlamydia trachomatis* (CT) and *Neisseria gonorrheae* (NG*)* NAAT Method (FOR A KS-IPP STUDY ONLY--CONTACT 785-368-8324 BEFORE SENDING SPECIMEN)

Collection instructions are printed on each collection swab kit. Please follow the instructions carefully and **only use collection kits compatible with the assay in use** at the Division of Health and Environmental Laboratories. Clearly label each CT/GC detection transport system with the patient's name (first and last) and date-collected,

then place a bar code from the Universal Requisition form used on the swab outer tube. Only the following two types of specimens are acceptable.

A. Male genital: Urethral specimens are collected in same manner as described for culture specimens. Use a collection kit swab distributed by the manufacturer of the assay kit being used by the Division of Health and Environmental Laboratories. Refrigerate specimens until pooled specimens groups are submitted to DHEL.

B. Female urogenital: Urogenital specimens are collected in the same manner as culture specimens. However, an assay specific collection swab must be used that corresponds with the assay kit used at the Division of Health and Environmental Laboratories. Refrigerate specimens after collection until shipment as a pooled group to DHEL.

C. Male or female urine specimens are accepted from KS-IPP selected sites: Patient should not have urinated for at least 1 hour prior to specimen collection. Collect specimen in a sterile plastic preservative-free specimen collection container. The patient should collect the first 15-20 mL of voided urine, NOT midstream. Testing sites will be provided with urine stabilizing screw cap tube transport systems. Refrigerate specimens until shipment to DHEL, arrival at DHEL must be within 10 days of specimen collection.

D. NO OTHER SPECIMENS ARE ACCEPTABLE FOR NUCLEIC ACID DETECTION METHOD AT THIS TIME.

The collection kits are stable for at least one year at room temperature prior to use.

Follow the collection information printed on each collection kit exactly as stated. The following is a summary of the swab collection method.

1. Remove excess mucus from the endocervix with the larger cleaning swab.
2. Snap apart the capped swab from the transport system and place the swab into the endocervical canal until most of the tip is no longer visible. Rotate the swab for 15-30 seconds in the endocervix to assure adequate adsorption by the swab.
3. Avoid touching any vaginal surfaces upon withdrawal of the swab to minimize contamination.
4. Return the collection swab to the transport system tube and snap them together and label the system with patient's name, date collected and facility.
5. Refrigerate as soon as possible.
6. No gel packs are needed during shipment of these specimens.

SHIPMENT

Shipment should occur by the next day after collection, since specimens must arrive at DHEL within 6 days of specimen collection (i.e., collected Wednesday with arrival by the following Monday). The specimen collection system should have the patient's name and date of collection written on it and a bar code from the Universal Requisition from the appropriately completed requisition sheets. Make sure each specimen tube top is tightly closed. Then place these tubes into a zip bag. The new transport system is about 6 inches long. Please place the completed requisition sheets into the pouch of the zip bag to keep them away from any moisture. Then place this bag on top of the padded specimens inside the box or in a padded envelope.

REPORTING PROCEDURE

Analysis of specimens for both ***C. trachomatis*** and ***N. gonorrhoeae*** will be performed each work day of the week. Reports are generated and mailed the following work day. The current NAAT uses the Strand Displacement Amplification (SDA) method for detection of the DNA of either of the two pathogens. Positive assays are those which exceed the MOTA score for the sample to the predetermined cutoff for that day's run, while negative assays fall below that cutoff value. Confirmation is not required. Indeterminants occur occasionally with specimens that contain excess mucous, making it very important to carefully clean the cervix before taking an endocervical or vaginal specimen.

REFERENCES

Schacter, Julius, and Chandler R. Dawson. 1978. Human *Chlamydia* Infections. PSG Publishing Co., Inc.

Wentworth, B.B., Judson, F.N., and Mary J.R. Gilchrist, eds. 1991. *Laboratory Methods for the Diagnosis of Sexually Transmitted Diseases* (2nd Edition).
American Public Health Association.

Vlaspolder, F. et. al. *Comparing Culture with DNA Probe Assay for Diagnosis of Gonococcal Infection.* Journal of Clinical Microbiology. Vol. 31, pp. 107-110, 1993

NON-SYPHILIS SEROLOGY

INTRODUCTION

Infection with bacterial, fungal, parasitic, rickettsial, and viral agents can often only be confirmed by antibody response in the patient. Isolation of a suspected agent is not always possible due to improper timing of specimen isolation, transport instability of agent, or contamination of the specimen. Even after isolation of an agent, its role in the disease may be questioned in the absence of a corresponding serological response. Paired acute and convalescent sera are required for confirmation of a current infection. The following table shows the serological tests used for the agents listed. (For rabies antibody test, see Appendix B, see page 56 for Rubella screening and diagnostic assays and see page 59 for serology tests performed at CDC.)

SEROLOGICAL TEST METHODS

Types of Disease/Agent	Etiological Agent	Test Method(s)
Neurotropic	Measles Mumps Enterovirus, ECHO, Coxsackie-virus	ELISA, IFA ELISA, IFA NT (on isolates only)
Arboviral	SLE, WEE, and WNV	ELISA (IgM &IgG) see p. 55 and 59
Respiratory	Hantavirus Enterovirus, ECHO, Coxsackievirus	ELISA, (IgM/IgG) see p.61 IFA (see p. 55) NT (on isolates only)
Rash, Maculopapular	Enterovirus Rubella (antibodies in young children peak in 3-6 weeks after onset) Measles	NT (on isolates only) ELISA (see p.56) ELISA, IFA
Vesicular	Coxsackie and Echovirus Varicella-Zoster	NT (on isolates only) ELISA, IFA
Rickettsial	Murine typhus R. rickettsii (RMSF)	IFA (IgG, IgM) IFA (IgG, IgM)
Hepatitis	Hepatitis A * Hepatitis B surface antigen ** Hepatitis C ***	ELISA-IgM ELISA ELISA-IgG

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* Testing for HAV IgM is only provided after consultation with Epidemiological Services at 877-427-7317.

** Testing of HBsAg is provided for health departments and State-supported health facilities. Please call the laboratory if you have any question.

*** HCV testing is only available at HIV CT Sites for IV Drug Abusers requesting HIV testing.

SPECIMEN COLLECTION, IDENTIFICATION, AND SHIPMENT

Clearly label each specimen with the patient's name or ID number and collection date. A Non-Syphilis Serology form should be completed and submitted with specimen(s). All information requested must be included, otherwise the specimen will not be tested until sender has provided missing information. These specimens will be returned or discarded if the sender does not comply with this request within one month.

A. Acute and Convalescent Bloods: Collect acute blood as soon after onset of symptoms as possible, but no later than seven days after onset. If storage is possible, the acute serum should be held frozen for submission with the convalescent specimen. Do not freeze whole blood. If serum cannot be separated at your facility, send whole blood specimens in a non-refrigerated mailer immediately.

B. Spinal fluids: Antibodies are rarely detected in spinal fluid, but may be detected in cases of encephalitis or other central nervous system diseases. When present, these antibodies have diagnostic significance. Antibody titers are commonly much higher in serum than spinal fluid. Acute and convalescent serum specimens should be tested along with the spinal fluid. Collect spinal fluid 10 to 14 days after onset. Take particular care to avoid contamination with blood on collection. Submit 1 to 3 ml in a sterile, screw-cap tube. Tape the cap for shipment and send on ice or gel pack.

C. Congenital Infections: Diagnosis of congenital infections requires serum specimens from both the infant and mother as soon after birth as possible. Cord blood is not appropriate. It is also necessary to submit a further infant's serum specimen two to six months later. Detection of specific IgM antibodies may be possible in the first specimen. The latter specimen will demonstrate either a drop in maternal-derived IgG antibodies or persistence of the elevated antibody titer in an infected infant.

D. Single Convalescent Serum: These specimens have very limited use except in cases where disease is not usually found in the general population. A specific diagnosis and complete history must accompany these specimens. (Residual low level antibody titers are not uncommon and can last for several years after an initial infection.)

REPORTING PROCEDURE AND INTERPRETATION

Analysis time is usually from four to ten days. Serological confirmation of a suspected disease is based upon the demonstration of a four-fold or greater rise in antibody titer between acute and convalescent serum specimens tested simultaneously in the same test run, or in the case of ELISA procedures, a diagnostic OD ratio for the specific assay.

Failure to demonstrate a rise in antibody titer does not rule out infection with an etiological agent. Many variables are associated with such test methods including test sensitivity, therapy, acute specimen collected too late, or convalescent specimen collected too early in the course of the disease. Results reported as less than the lowest dilution tested do not preclude the presence of antibody, but rather indicate that under the conditions and testing methods used, antibodies were not detected.

Nonspecific or anticomplementary reactions which invalidate test results occur occasionally. Causes of such reactions include bacterial contamination of serum, chemically contaminated containers, and inherent serum properties found particularly among elderly or immunosuppressed individuals. Chances of microbial contamination can be reduced if sera held beyond 48 hours are frozen rather than refrigerated. A new specimen must be submitted for testing when nonspecific or anticomplementary reactions occur.

REFERENCES

Murray, P. R. et. al. (Ed.) 1999. *Manual of Clinical Microbiology*, 7th ed. American Society for Microbiology, Washington, D.C. 20005.

RUBELLA IMMUNE STATUS

Prevention of congenital rubella infections is a public health priority. Nosocomial rubella transmissions are a major source of these infections; therefore, we strongly concur with policies currently advocated by the American Hospital Association and the U.S. Public Health Service concerning employee immunizations. As stated by the latter agency, both female and male employees who might contract rubella from infected patients or who, if infected, might transmit rubella to pregnant patients or to pregnant coworkers should be vaccinated against rubella unless there are contraindications. The rubella vaccination of a woman who is not known to be pregnant and has no history of vaccination is justifiable. Serological testing is not warranted prior to or after these vaccinations. Accordingly, we discourage routine screening of health care employees and do not offer immune status tests for such groups.

Selective rubella screening is available to Kansas residents through local public health laboratories (e.g., when there is a question of pregnancy, a recent exposure to rubella and no record of vaccination or a prior positive immune status assay response). An Enzyme-linked Immunosorbent Assay (ELISA) is performed on single serum specimens for such screening assays, with the results given as a positive or negative response.

RUBELLA DIAGNOSTIC TESTING

ELISA tests can be used to determine whether an individual has recently been exposed to rubella virus. Evaluations are limited to persons exhibiting a rash illness, pregnant women following exposure to rubella without known seropositive PHA or equivalent test result, and suspected congenital rubella syndrome cases.

For the first two patient groups, an acute serum must be obtained within a week after exposure and a convalescent specimen drawn two to three weeks after exposure or rash. A positive diagnostic OD ratio, while considered diagnostic for exposure, does not distinguish between re-exposure of immune individuals (natural or vaccine induced) and primary rubella infections. However, high levels of IgM specific antibody are usually detectable for up to 30 days after primary rubella infections. Currently such testing is available at CDC for requests that meet their criteria. Please call the state laboratory before sending such specimens, since CDC requests must be referred through this facility.

Congenital rubella diagnosis requires serum specimens from both the infant and mother (**NOT cord blood**) no later than the first week after birth. If a positive ELISA or equivalent test result occurs, a second serum is required from the baby at an age of four to six months by which time maternal antibodies (IgG) have normally declined. IgM in infected infants can be detected for up to six months after birth. IgM detection beyond six months may indicate a postnatal infection. CDC requirements must be met for such evaluations. Please contact the Bureau of Epidemiology before sending requests for IgM testing (877-427-7317).

SPECIMEN COLLECTION, IDENTIFICATION, AND SHIPMENT

The same procedures for labeling the specimen tube and for blood collection should be used as have been outlined for non-syphilis serology on page 54 including a completed Universal Request form.

Mailing kits are available with 5 tube multimailer containers (see LIST OF SPECIMEN KITS AVAILABLE, page 24). Testing will be done once a week.

REFERENCES

Preblud, S.R., H.C. Stetlen, and J.A. Frank, et al. 1981. *Fetal Risk Associated with Rubella Vaccine*, JAMA 246:1413-1417.

Murray, P. R. et. al. (Ed.) 1999. *Manual of Clinical Microbiology*, 7th ed. American Society for Microbiology, Washington, D.C. 20005.

HEPATITIS SEROLOGY

INTRODUCTION

Hepatitis B serology assays are available on a limited basis for diagnosis of acute and chronic disease among clients of local health departments and some state-operated health care facilities. Additional emphasis is placed on testing prenatal patients and the household and sexual contacts of any prenatal clients that are confirmed HBsAG positive. Those contacts lacking hepatitis B antigen and antibody markers can be provided HBV vaccinations and tested for antibody response at an appropriate time. In addition, the neonates of those women positive for hepatitis B antigen can be given HBIG and the HBV vaccine series starting at birth and followed-up for seroconversion status at one year of age.

SPECIMEN COLLECTION AND IDENTIFICATION

Only serum is acceptable for serological testing (e.g., either a red top blood tube or separated sera). All specimens must be accompanied by a fully completed hepatitis requisition form. The patient's name and date of specimen collection must be written on the specimen tube. Failure to perform these steps may significantly delay specimen analysis or even cause the specimen to be rejected.

POPULATION ACCEPTED FOR TESTING

Hepatitis B surface antigen (HBsAG) testing is available to the following patient populations: symptomatic patients; prenatal clients; refugees; sexual and needle-sharing contacts of known infected persons; individuals previously tested positive for HBsAG. **Testing for HBsAG is not provided to deal with workers compensation claims related to possible health care worker exposure to patient's bodily fluids**. In addition, seroconversion confirmation by anti-HBsAG assays following the HBV vaccination is only made available to vaccinated neonates and household contacts associated with HBsAG positive prenatal clients from local health departments. Confirmation testing will be performed at a contracted Reference Laboratory.

SPECIMEN REQUIREMENTS

A minimum of 3 ml of serum is required. Confirmation of positive HBsAG screening results is performed by a reference laboratory. Additional hepatitis B assay procedures may be performed at this reference laboratory based on predetermined patient criteria.

At this time, no routine hepatitis A or C procedures are performed at the Division of Health and Environmental Laboratories. Under special epidemiological circumstances, submission of specimens through the Division of Health and Environmental Laboratories to CDC may be possible. Please call (785-296-1620) before submitting any specimens for other hepatitis viral agents.

Multiple mailers bottles with outer box, Universal Requisition forms and blood tubes are available to health departments and some state-operated health care facilities through the mailroom at DHEL. See the SPECIMEN KIT SECTION on page 24 for additional information.

SEROLOGICAL TESTS REFERRED TO THE CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) THROUGH TO DIVISION OF HEALTH AND ENVIRONMENTAL LABORATORIES

INTRODUCTION

Serological tests for antibody or antigen detection of many bacterial, fungal, parasitic, rickettsial, and viral agents not performed at this laboratory are available from CDC. Specimens must be submitted through the Division of Health and Environmental Laboratories, Virology and Serology Laboratory, in the same manner as any non-syphilis serology serum or cerebral spinal fluid specimen.

SPECIMEN COLLECTION AND IDENTIFICATION

All specimens submitted to the Division of Health and Environmental Laboratories to be forwarded to CDC must include a completed CDC form 50.34. The CDC forms and instruction packet are available through our Sample and Data Management Office. For further information and additional forms, please call (785) 296-1620. The turn-around time for results from CDC is approximately at four weeks. Call the BEDP at 1-877-427-7318 before sending specimens to DHEL.

Properly collected paired acute and convalescent sera are required for most serological testing. However, CDC will accept single sera for testing of parasite and fungal diseases only, as elevated antibody titers can be diagnostic for some of these agents. For certain tests, CDC requires additional patient information before tests will be performed. These include:

A. Epstein-Barr virus antibody testing can be of diagnostic value only under certain conditions due to the lifelong persistence of the antibodies. CDC will only test specimens for EBV if:

- Suspected IM with neurotropic involvement, or cases of unusual public health importance.
- Serum specimens from possible outbreaks of IM or EBV infection.

All requests for EBV tests must be approved by prior consultation with the appropriate CDC laboratory before specimens are sent for testing. Paired sera and a complete patient history must accompany all specimens submitted. The history should include age and sex of the patient, date of onset, clinical diagnosis, associated illness, results of previous EBV and IM serology, and other appropriate clinical data.

B. Malaria antibody testing is performed for:

- A patient with a febrile illness who is strongly suspected of having malaria and from whom blood slides are repeatedly negative for parasites.

- Donors to a patient who developed malaria following a blood transfusion.
- Standardizing control sera from laboratories that have, or are establishing, programs to test for malaria antibodies.
- Other special situations that are approved in advance by the chief or assistant chief of the Malaria Branch at CDC.

A properly collected, stained, and examined blood smear is the best way to diagnose acute malaria; therefore, a slide should be sent to CDC through the Division of Health and Environmental Laboratories when diagnosis is in doubt at both local and State levels.

Information on the CDC form 50.34 should include whether a blood slide was collected and examined for malaria parasites, the results, including species and parasitemia density if possible, and whether a transfusion was given or not.

Unacceptable specimens include:

- Routine testing of well persons having had possible malaria contact overseas.
- Routine screening of certain populations (refugees, immigrants, etc.) for evidence of previous exposure.
- Confirmation of the species of a parasite on a blood smear or otherwise suspected.
- Completion of a clinical record on a patient known already to have malaria and who has recovered.

C. Serological tests for schistosomiasis, toxoplasmosis, and amebiasis:

1. *Schistosoma sp.*

- Serum specimens must be from a patient with an illness compatible with urinary, intestinal, and/or ectopic schistosomiasis whose urine, stool, and/or tissues are negative for schistosomes after careful microscopic examination of one or more specimens by the primary laboratory or referring state laboratory.
- Cerebrospinal fluid specimen and, if possible, serum specimens should be obtained and forwarded from patients with known or suspected central nervous system schistosomiasis; prior arrangements are advised.

2. *Toxoplasma gondii*

- Screening is no longer provided because there are commercially available kits and reagents for this purpose.
- Serum specimens will be accepted when accompanied by a request for confirmation of previous data, testing for IgM antibodies, and testing known or suspected AIDS patients.

For each of the above, previous serologic testing data must be included in the CDC form 50.34.

3. *E. histolytica*

- Serology specimens from suspected intestinal amebiasis must indicate dates and results of laboratory and clinical examinations. In particular, the number, type (i.e., direct, concentration method) of stool examinations, and results of any other direct parasitologic examination (i.e., biopsy) must be stated.
- Serology specimens from suspected extraintestinal amebiasis must be accompanied by a summary of supporting clinical, radiographic, and laboratory data.

D. Pneumococcal antibody assays are limited to patients who have developed invasive pneumococcal infections following immunization with the pneumococcal vaccine.

E. Rabies serological testing is only available to persons vaccinated with DEV or those who are suspected of being immunocompromised (see Appendix C).

Neck biopsy and saliva specimens are accepted for human rabies diagnosis.

F. Arbovirus serological testing is available if acute and convalescent sera and CSF (collect during the first week after onset) can be provided. An ELISA antibody-capture method for detection of IgM and IgG antibodies is utilized to determine recent arbovirus exposures.

Brain biopsy and CSF (2-5 ml) are acceptable specimens for human cases of viral encephalitis.

G. Lyme disease serological testing is provided under unusual circumstances and requires a completed **Lyme Disease Report form (available from the Virology Laboratory)**, acute and convalescent serum specimens, and a completed CDC form 50.34. Testing is only provided for complicated cases such as for an immunocompromised patient.

H. Cat Scratch Disease (C.D.) *Bartonella henselae* and its association with bacillary angiomatosis (BA) is currently being investigated at CDC. A serum specimen (3-5 ml) is required along with a completed 50.34 form for forwarding to CDC. The study population should be limited to immunocompromised patients and to individuals with unusual symptoms.

I. Hantavirus IgM and IgG serological testing and analysis of follow-up sera and tissue specimens are performed at CDC. For suspected hantavirus infections

and to receive the Hantavirus Disease Report forms required by CDC, please call (785) 296-1620. The
BEDP must be contacted at 1-877-427-7318 before sending specimens to DHEL.

J. Serological testing for Brucella, Tularemia, and Leptospirosis are available at CDC for difficult or unusual cases. CDC must agree to any testing prior to submission of serum specimens to DHEL. Please call the Serology Laboratory at (785) 296-1620.

SYPHILIS SEROLOGY

INTRODUCTION

The reagin test performed in the Division of Health and Environmental Laboratories is the RPR. The RPR test will be performed on all serum specimens received for syphilis screening. Any reactive specimen will be quantified to an end-point titer.

The FTA-ABS-DS test is performed on serum only in the following situations.

- Reactive reagin test on premarital or prenatal specimens. (Kansas no longer requires a premarital serology, but we do perform serologies for out-of-state marriages.)

- Two reactive reagin tests* (same test performed two to three weeks apart) with no clinical symptoms or history suggesting syphilis. Please provide results of the first reagin test.

  *While it is desirable to have two reactive reagin tests separated by an interval of time, an FTA-ABS-DS test will be performed upon request, when a single reactive test can be confirmed by repeat test in our laboratory.

- Non-reactive reagin test with symptoms or history suggesting late syphilis (cardiovascular or neurological involvement). Physicians must provide appropriate clinic information and check off the diagnosis box on the requisition form.

- Specimen requests for FTA-ABS-DS testing in patients who are suspected of possible late syphilis that test negative for RPR and are FTA-ABS-DS positive will be forwarded to CDC for reference FTA-ABS-DS testing. The submitter will be notified by phone about the specimen status when the specimen is forwarded. A report will be issued after results are received from the CDC.

- Exceptions to this policy must be discussed with the Serology Unit, (785) 296-1620, prior to submission of specimen. Please note that FTA-ABS tests on spinal fluids are offered in the Division of Health and Environmental Laboratories when neurological syphilis is suspected in patients with a positive FTA-ABS-DS serum result. The Centers for Disease Control will accept FTA-ABD-DS positive spinal fluids for confirmation testing. Along with the CSF they require a sera specimen and a complete patient history. The spinal fluid must be free of red blood cells and blood sera contamination in order to give valid results.

SPECIMEN COLLECTION

A. Serum. Submit 2 ml of serum or 5 ml of whole, clotted blood. Plasma is unsatisfactory. Blood or serum specimens which are contaminated with bacterial growth, which contain a gross amount of chyle (the milky, white, fatty fluid), or are hemolyzed, are unsatisfactory. Cord blood is an unsatisfactory specimen for screening for syphilis in the newborn. Cord blood would indicate only maternal antibodies.

B. Cerebrospinal Fluid. Aseptically collect 1 to 2 ml CSF and submit in any sterile tube. CSF contaminated with blood is unsatisfactory as the test would possibly detect serum antibody rather than antibodies of the CSF. CSF which is bacterially contaminated is unsatisfactory.

SPECIMEN IDENTIFICATION AND SHIPMENT

A. Be sure the patient's name is on each tube submitted. Completely fill out a Universal Requisition form, indicating any previous reactive results.

B. **CLIA regulations require that patient name, attending physician, facility address, and date of collection must be completed on the requisition form before the specimen can be tested. Incomplete data will delay testing, as these requisition forms are returned to the submitter for completion and specimens are held until the form returns. Information not received in 45 days results in specimen being discarded.**

TESTING AND REPORTING PROCEDURES

TEST RESULTS		INTERPRETATION
RPR	FTA-ABS-DS	
Reactive	Reactive	These results usually indicate syphilis
Reactive	Nonreactive	"Biological False Positives" (BFP) reaction in reagin tests may be caused by infections, immunizations, inflammatory disease, immunity abnormalities, drug addiction, pregnancy, or aging. Tests should be repeated on a follow-up specimen if doubt exists.
Reactive	Not Done	Refer to FTA-ABS-DS policy in Introduction.
Reactive	Previous Date	Patient has been tested positive by FTA-ABS-DS on that date and will remain positive for an indefinite period of time, possibly life.
Reactive	Reactive (1+) minimal	If this is the first specimen, submit another. If this is the second specimen with a 1+ result, diagnosis will rest on clinical evidence.

Reactive	Atypical Staining	FTA-ABS-DS does not show proper staining pattern. Please submit another specimen.
Nonreactive	Not Done	Treponemal tests are not indicated unless late syphilis is suspected according to clinical data.
Nonreactive	Reactive	This result usually indicates previously treated syphilis or late syphilis (untreated).

C. Use syphilis (white label) mailers. Put return address on the outside of the mailer and ship as soon as possible.

The serum RPR is routinely performed the same day serum specimens are received. Spinal fluids are held until Tuesday and then tested by FTA-ABS-DS. All reactive specimens are sent to CDC for validation of the reactive FTA-ABS-DS determinations that may indicate possible neurological syphilis.

Darkfield examination can be made by arrangement with the Sexually Transmitted Disease Program, (785) 296-5598.

Analysis Time: (excluding mailing times)

Serum RPR - 24 hours
Spinal FTA-DS-ABS - 1 to 5 days
FTA-DS-ABS - 1 to 5 days

REFERENCE

Larson, Pope, Johnson and Kennedy. *A Manual of Tests for Syphilis*. 9th Edition. American Public Health Association. 1998.

VIRAL ISOLATION

INTRODUCTION

Information obtained from virus culture is designed to provide meaningful data to help support or confirm the physician's clinical observations. Failure to isolate a virus from clinical material does not necessarily mean that the suspected agent is absent or that the diagnosis is incorrect.

SUGGESTED CLINICAL SPECIMENS FOR VIRUS CULTURE

Infection/Syndrome	Etiological Agent	Specimen of Choice[1]	Transport Medium Required
Central Nervous System (CNS)	Enterovirus, (i.e., Coxsackie, Echo, Polio)	CSF[2] Feces[4] Throat Swab	- + +
	Mumps	Throat Swab Urine	+ -
	Herpes Simplex (HSV)	Throat or NP[3] Swab Vesicle fluid or Swab CSF	+ + -
Congenital Infections	Cytomegalovirus (CMV)*	Throat or NP Swab Urine	+ -
	HSV	Lesion scrapings (same as above except no urine)	+ +
Food borne illness	Calicivirus-like agents	Feces in modified Cary Blair	+
Rash, maculopapular	Adenovirus	Feces Throat Swab	+ +
	Enterovirus	Feces Throat Swab	+ +
	Measles (Rubeola) Rubella	throat Swab Throat Swab Urine	+ + -
Rash, vesicular and muco-cutaneous ulcers	Coxsackie A, Echo	Feces Throat Swab Vesicle Fluid or Swab of Lesion	+ + +
	HSV	Vesicle Fluid or Swab of Lesion	+
	Varicella Zoster (VZV)	(Same as Above)	+
Respiratory	Adenovirus, Enterovirus, Influenza, Parainfluenza, Rhinovirus	Throat or NP Swab	+
	Respiratory Syncytial Virus (RSV)*	Throat or NP Swab	+

1. If specimen(s) cannot be delivered to the laboratory within 72 hours of collection, freeze immediately after collection and hold at lowest temperature available pending shipment with dry ice (see page 67).
   **However, if either CMV or RSV are the suspected etiological agents, do not freeze the specimen as such treatment destroys these viruses. Maintain such specimens at or near 4°C from isolation through shipping.*
2. CSF specimens should be frozen ASAP and submitted on dry ice if more than 24 hours is required for transport to DHEL.
3. Small stool specimens of less than five grams and rectal swabs (less desirable) should be immediately placed in viral transport medium (VTM) as described later in this section.

Numerous factors can account for this isolation failure; improper time of collection of the specimen (virus no longer being shed), improper storage or transport of the specimen, use of inadequate transport medium, or the absence of a sufficiently sensitive laboratory procedure. The importance of appropriate acute and convalescent serum specimens for supporting the viral diagnosis cannot be over stressed.

Specimens for virus culture should be collected as soon as possible after onset of illness. See previous table for culture services available, and sources from which to obtain specimens.

SPECIMEN COLLECTION AND IDENTIFICATION

Clearly label each specimen with patient's name or ID number, type of specimen, and collection date. Complete a Universal Form to be sent with the specimen. Compliance with these requests will result in the most rapid, effective response by this laboratory.

A. Autopsy or biopsy. Collect fresh, unfixed tissue from the suggested sites involved with separate sterile instruments for each sample. Place each specimen into a separate small vial containing VTM*. Store the specimens at 4°C pending shipment.

B. CSF. Aseptically collect 1 to 2 ml of CSF and transfer to a sterile tube.* Store until shipment at 4°C if less than 24 hours required for delivery to DHEL (use ice packs); otherwise, freeze immediately and send on dry ice.

C. Feces. A specimen of 4 to 8 gm (2 cm across) should be placed in a sterile, screw-cap jar (see LIST OF SPECIMEN KITS AVAILABLE, VIRAL SPECIMENS). For
Calicivirus-like agents use suspend in modified Cary Blair medium.

Smaller specimens of feces and rectal swab specimens should be placed in VTM. For swab specimens, insert a dry cotton swab at least 5 cm into the rectum, rotate the stick, and then withdraw it. Some fecal material should be visible on the cotton. Break swab off into the VTM and screw cap on tightly.* Keep cold (4°C) pending shipment.

D. Tissue and lesion scrapings: Suspend these specimens in VTM in a sterile, screw-cap tube.* Store at 4°C pending shipment.

E. Throat swab: Carefully rub the posterior pharynx, inflamed and erythematous areas, or visible lesions with a dry, sterile, cotton swab or viral culturette swab. The swab should not touch the tongue or buccal mucosa. Break off the swab into a tube of VTM or return swab to the culturette holder for the respective collection systems.* Store at 4°C pending shipment.

F. Urine: For the maximum recovery of virus, collect several clean-voided urine specimens on successive days. Each specimen should be placed in a sterile, screw-cap tube and shipped on the day collected with ice packs*.

G. Washings, bronchial or nasopharyngeal: Collect in a sterile screw cap vial containing VTM.* Store at 4°C pending shipment.

*Tubes and bottles used should be tightened after filling and tape placed around the lid to eliminate leakage of contents during shipment.

SHIPMENT OF SPECIMENS

Keep the specimen cold from the time of collection through the delivery to the laboratory. If delivery is not possible within 72 hours, the specimen should be frozen promptly, except for CMV and RSV specimens which should never be frozen. Frozen specimens should be shipped to the laboratory on dry ice (4 to 5 lbs.). Do not freeze and then send with ice packs only; just keep specimen at 4°C during storage and shipment.

Suggested Shipment of Specimens (ice packs or dry ice):

- Place labeled specimens, with caps screwed tight and taped, in a plastic zip bag and seal.
- Place completed Universal Form in the zip bag outer pouch and seal.
- Put two or more cold packs in a pre-cooled Styrofoam container. Use 4 to 5 lbs. of dry ice instead if specimens must be frozen.
- Pack specimens with sufficient padding to prevent shifting during shipment, remember that dry ice will sublimate in transit.

For critical specimens, please notify the laboratory, (785) 296-1620, of the method of shipment and expected delivery time.

REPORTING PROCEDURES AND INTERPRETATION

Analysis may require from one week to over one month depending on the specimen. As alluded to previously in the Virology "Introduction," failure to isolate an agent does not rule out the agent as the etiological virus. Conversely, since patients may asymptomatically carry a variety of viruses, some resulting isolates may be unrelated to the current clinical illness. Serological confirmations are used to clarify such questions. Isolates from CSF, although they are rather rare occurrences, are considered to be diagnostic.

Positive Report: will have the **virus** isolated written in the laboratory report section of the specimen form.

Negative Report: will have "**no virus found**" written in the laboratory report section of the specimen form.

REFERENCES

Lennette and Schmidt. 1979. *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*. 5th Edition. Washington, D.C.: American Public Health Association.

COMMON VIRAL SPECIMEN REQUESTS AND SPECIMEN REQUIREMENTS

Virus	Specimen choice based upon symptoms	Isola-tion	Typ-ing	Methods Used
Adenovirus	Conjunctival or throat swab	✓	✓	Cell culture; NT; DFA
MV	Urine (5ml), heparin blood tube buffy coat, throat swab	✓		Cell culture; DFA
Enterovirus Echo Coxsackie Polio	Throat swab, stool or rectal swab, CSF (2ml), Brain biopsy, pericardial fluid	✓	✓	Cell culture; NT
HSV	Genital swab, swab of lesion/scraping, CSF (2ml), brain biopsy	✓		Cell culture; DFA
Influenza	Throat swab or washing nasopharyngeal swab	✓	✓	Cell culture; Hen's eggs; HA/HAI; IFA
Mumps	Throat swab, urine (5ml) CSF	✓		Cell culture; IFA
Parainfluenza	Throat swab or nasopharyngeal swab	✓	✓	Cell culture; NT; DFA
RSV	Throat swab or nasopharyngeal swab	✓		Cell culture; DFA
Rubeola (Measles)	Throat swab or washing, nasopharyngeal swab	✓		Cell culture; IFA
VZV	Vesicle scrapings	✓		Cell culture; DFA
Caliciviruses	Fresh stool in modified Cary-Blair	✓		PCR

VII. ENVIRONMENTAL CHEMISTRY

TECHNICAL INFORMATION

BLOOD LEAD SCREENING

INTRODUCTION

Lead contamination is ubiquitous in our environment. Blood lead concentrations exceeding 10 □g/dL can pose health risks, particularly in children. In order to measure blood lead concentrations at or below this level, special precautions must be taken to avoid contaminating the specimen. Lead screening procedures can be performed on properly collected capillary filter paper or venipuncture specimens. Repeat positive screening tests (20 □g/dL or more) must be confirmed using venipunture techniques.

Blood lead screening samples may be submitted by any health care facility that has a valid facility ID. Facility IDs can be obtained by calling the laboratory at (785)296-1620. The majority of lead screening is done for children six years of age or younger. However, there are exceptions such as expectant mothers and children living in high risk areas.

SUPPLIES AND EQUIPMENT

Blood lead screening kits are currently being supplied to all facilities, with a valid facility ID, free of charge. Facilities may use their own venipuncture purple top tubes which contain EDTA added for a preservative.

A. Department of Health & Environmental Laboratories (DHEL) lead screening kit
    a. Blood Lead Submission Form (with filter paper attached)
    b. Microflow lancet (available upon request)
    c. Filter paper spot template, used to judge if the blood spot is large enough to analyze (available upon request)
    d. EDTA purple top tube used for venipuncture confirmation (available upon request)
B. Facilities are responsible for the following supplies
    a. 10% bleach solution prepared within the last 24 hours
    b. Paper towels (do not use recycled products)
    c. Alcohol swabs
    d. Gauze squares
    e. Adhesive bandages
    f. Biohazard waste container
    g. Sharp disposal container

QUALITY CONTROL

Do not use filter paper that has been wet, wrinkled, or exposed to dust contamination. Do not substitute another type of collection tube and do not use the vacutainer beyond the expiration date on the outside of the tube.

COLLECTION PROCEDURE

A. Filter paper collection Instructions can be found on the inside flap of the Blood Lead Submission Form.
B. Thoroughly clean the bench top or collection area using a bleach solution.
C. Determine whether a finger, toe, or heel puncture is appropriate for the child. Puncturing the heel is preferable when the child is less than one year old, if the heel is large and cannot be securely grasped, use the great toe. Do not select the foot as puncture site once the child has begun to walk. For older children select the middle or fourth finger.
D. Thoroughly wash the hand or foot of the child using mild soap and plenty of warm water. Be certain that no soap residue remains on the skin. The puncture site should be pink or warm to the touch. Continue to warm the site with water until this is accomplished.
E. Put on your gloves.

HEEL PUNCTURE

A. Fold back the wrap around cover before collecting filter paper blood spot.
B. Grasp the foot around the heel with the palm of your hand on the bottom of the child's foot and your thumb and index finger around the heel.
C. Wipe the area with the alcohol swab and dry with the gauze square.
D. Place the lancet device firmly against the heel in the proper region. Depress the plunger using your index finger and release immediately. Discard the lancet in the sharps container.
E. Use the gauze square to wipe away the first drop of blood which forms.
F. Gently touch the filter paper to the second drop of blood. Allow blood to soak through completely filling the preprinted circle. The blood spot should be at least the size of a quarter inch punch.
G. Fill each of the three circles with a single drop of blood. Do not layer successive drops.
H. Stop the blood flow by applying pressure with the gauze. When bleeding has stopped apply an adhesive bandage.
I. Dispose of gauze, gloves, and other soiled materials by placing them in a biohazard bag or container.
J. Allow the blood spots to air dry for at least 2 hours before covering them with the wrap around cover.
K. Use the address label, provided on the submission form, and mail the submission form (with filter paper specimen attached) to KDHE.
L. Multiple specimens may be mailed in the same envelope as long as the blood spots are thoroughly dried. Stack the filter papers by alternating the blood spots on opposite ends (if the blood spots are on the right side on the envelope the next form should be rotated so the blood spots are on the left side of the envelope).

FINGER OR TOE PUNCTURE

A. Fold back the wrap around cover before collecting filter paper blood spot.
B. Grasp the child's finger (or toe), placing your fingers over the distal joint.
C. Wipe the area with the alcohol swab and dry with the gauze.
D. Place the lancet device firmly against the finger tip (or toe) in a vertical position on the outer third of the finger pad. Depress the plunger using your index finger and release immediately. Discard the lancet in the sharps container.
E. Use the gauze square to wipe away the first drop of blood which forms.
F. Gently touch the filter paper to the second drop of blood. Allow blood to soak through completely filling the preprinted circle. The blood spot should be at least the size of a quarter inch punch.
G. Fill each of the three circles with a single drop of blood. Do not layer successive drops.
H. Stop the blood flow by applying pressure with the gauze. When bleeding has stopped apply an adhesive bandage.
I. Dispose of gauze, gloves, and other soiled materials by placing them in a biohazard bag or container.
J. Allow the blood spots to air dry for at least 2 hours before covering them with the wrap around cover.
K. Use the address label, provided on the submission form, and mail the submission form (with filter paper specimen attached) to KDHE.
L. Multiple specimens may be mailed in the same envelope as long as the blood spots are thoroughly dried. Stack the filter papers by alternating the blood spots on opposite ends (if the blood spots are on the right side on the envelope the next form should be rotated so the blood spots are on the left side of the envelope).

PROCEDURE NOTES

- Fill out the submission form completely before collecting the specimen. Forms sent to KDHE containing missing information could lead to a delay in analysis or reporting of blood lead results.
- The blood sample for lead analysis must be collected before any samples for other purposes to assure that contamination of the collection site by reagent from other tests does not occur. Contamination can lead to falsely high screening results.
- Squeezing of the heel, toe or finger will introduce interstitial fluids into the specimen. This dilutes the specimen and can cause a falsely low test result.
- Alcohol left on the puncture site will dilute the specimen and cause hemolysis. This can produce a falsely low result.
- Wipe away blood which collects during the time between specimens (different test) with the gauze. Platelets tend to collect at the edges of the puncture and may cause clots to form.
- Avoid the use of cotton or rayon balls in place of the gauze. These fibers may cause the formation of clots, reducing the blood flow.

    - If blood flow is not adequate check for the following errors:
        - The puncture site was not warmed properly.
        - The finger, toe or foot is at a level above the child's heart.
        - Excessive pressure or squeezing is restricting blood flow.
        - The lancet was not placed firmly against the skin before depressing the plunger.
- Do not layer multiple blood spots on top of each other. This could lead to false results. Multi spotted specimen will be flagged with a comment on the report.
- Not allowing the blood spots to completely dry can cause the spreading of blood onto the coverlet and the possibility of false results. Blood spots not allowed to dry will be flagged with a comment on the report.

TEST PROCEDURE

Blood lead samples are analyzed using an inductively coupled plasma mass spectrometer (ICP-MS).

LIMITATION OF THE PROCEDURE

This is a screening procedure. All repeatable positive screening tests must be confirmed using venipunture techniques.

INTERPRETATION OF RESULTS

A filter paper blood lead value less than 10 □g/dL is considered to be normal by CDC guidelines.

Filter paper blood lead values between 10 and 20 □g/dL are considered elevated, and clinical monitoring of the child is recommended.

Filter paper blood lead values greater than or equal to 20 □g/dL are considered to be positive, indicating a definite health risk. A follow-up venipuncture specimen is required for confirmation. This should be obtained using a venipuncture EDTA purple top tube.

Specimens may be rejected for the following reasons:

A. Blood clotted
B. Sample not identified (Missing Patient Information Sheet)
C. Quantity insufficient (blood spot not big enough to analyze)
D. Blood spot not allowed to dry completely
E. Blood spot appears to be diluted (puncture site not completely dried before collecting blood spots)
F. Venipuncture tube has expired
G. Wrong collection tube used

*Reports will be withheld until all required missing information is provided:

A. Date of collection
B. Physician's name
C. Patient identification
D. Submitter identification
E. Patient's date of birth

RESPONSE TO HIGH LEVEL BLOOD LEAD RESULTS

When blood lead values greater than or equal to 20 □g/dL but less than 45 □g/dL are detected, the lab will send a copy of the report by fax within 24 hours the result was obtained. The original report will be mailed.

When blood lead values greater than or equal to 45 □g/dL are detected, the lab will send a copy of the report by fax and a phone call will be made to let the facility know that a critical value was detected. The fax and phone call will be made the day the result was obtained. The original report will be mailed.

APPENDICES

APPENDIX A

Requirements for Handling and Shipping Laboratory Specimens

If you are sending:	Use this packing method:*	Use these outside labels:
Diagnostic Specimens** This would include any specimen from human patients such as blood, sputum, urine, tissue, etc.	• double wall mailing container (see Figure 1) • inner container should have absorbent material • secure primary containers with vibration resistant material (foam rubber, etc.)	• laboratory mailing label with "clinical specimen" appearing on label
Infectious Substance/Etiological Agent This includes all specimens known or reasonably believed to contain an infectious agent and all culture isolates sent for identification	• UN 6.2 certified infectious substance shipper. • secure cap of primary containers with tape.	• laboratory mailing label • biohazard symbol (see Figure 2) • send by registered mail with return receipt • notify lab of shipment
Other Hazardous Materials Shipping specimens or cultures with dry ice.	• Same packing requirements as above, plus • pack in a vented styrofoam cooler with dry ice wrapped in paper	• Use criteria above for type of specimen or culture • Also attach ORM-A form to alert shipper to presence of dry ice.

* Use of designated state laboratory mailers complies with these packing requirements.

** Defined I the Domestic Mail Manual as “not reasonably believed to contain an etiologic agent.”

References

Hazardous, Restricted, and Perishable Mail, Publication 52. July 1999. Section 346, Toxic Substances and Infectious Substances (Hazard Class 6).

Single I.D.S. System Small (Ambient)

1 Box
1 Bubble Sheet
1 Partition
1 Infectious Label
1 Pressure Vessel
1 Cap
1 Absorbent Disk

Single Diagnostic Kit (Ambient)

For Microbiology Specimens or CSF in Large Tubes

1 Box
1 Bubble Sheet
1 Absorbent Pad
1 Pressure Vessel
1 Cap

For Serum or Blood Tubes

Informational link at: www.inmarkinc.com/

APPENDIX B

RABIES

SPECIMEN COLLECTION

The laboratory procedure is performed on the intact brain; therefore, the animal should be euthanized in a manner that will not destroy the brain. Only the animal's head (or brain for large animals) should be submitted for diagnostic purpose.

Animal Species Considerations in Evaluating Rabies Risk

Some animals are much more likely to be infected with rabies virus than others. For example, carnivorous wild animals (especially skunks, raccoons, fox, coyotes, and bobcats), and bats are the animals most commonly infected with rabies. These species have been the cause of most of the human rabies in the United States since 1960. Unless the animal is tested and shown not to be rabid, prophylaxis should be initiated upon bite or non-bite exposure to one of these animals. (See definition in "Type of Exposure," below.)

The likelihood that a domestic dog or cat would be infected with rabies varies from region to region; hence, the need for post-exposure prophylaxis also varies.

Rodents (such as squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, and mice) and lagomorphs (including rabbits and hares) are rarely found to be infected with rabies and have not been known to cause human rabies in the United States; their bites almost never call for antirabies prophylaxis. Therefore, in these cases the state or local health department should be consulted before initiating post-exposure antirabies prophylaxis.

All questions should be directed to the Bureau of Disease Prevention and Health Promotion, Kansas Department of Health and Environment, Curtis Building, Topeka, Kansas, 66612. Call 877-427-7317

FOR: PACKING, IDENTIFICATION, AND SHIPMENT INFORMATION
KSU LINK.....Animal Heads
www.vet.ksu.edu/depts/rabies/fatest.htm

FOR: ANTIRABIES TREATMENT INFORMATION- PROPHYLAXIS and PRE-EXPOSURE
CDC LINK.....Exposure and treatment
www.cdc.gov/ncidod/dvrd/rabies/prevention&control/preventi.htm

FOR: RABIES ANTIBODY TESTING INFORMATION (Human and animal serology tests)

KSU LINK.....RFFIT testing

www.vet.ksu.edu/depts/rabies/rffit.htm

If you have additional questions, please call Rolan Davis at (785) 532-4298 or email him at RDavis@vet.ksu.edu